Name _____ Student number _____

University of Guelph
Department of Molecular and Cellular Biology

Summer 2016 BIOC*2580 Introductory Biochemistry

Midterm
Monday June 6, 2016 5:30 pm–6:45 pm RICH 2520

This exam determines 25% of the final course grade

Instructor: Dr. Enoka Wijekoon

Total Marks: 50 Total Time: 75 minutes Total pages in this booklet: 11

Instructions:

1. Print your full name and student number in the spaces provided at the top of each page.
2. This exam consists of 2 sections. Please attempt all questions.
3. Please answer the multiple choice questions in section 1 on the scantron card provided. Please answer section 2 in the space provided in the booklet.
4. Use only pen to answer the questions in section 2. Do NOT use red pen.
5. Hand in all materials including this exam booklet.

Scantron Instructions:

1. Use a soft black **lead pencil only** on the Scantron card and erase cleanly if needed. Do NOT use white-out or ink on the Scantron card.
2. Fill out your name and ID number on the score sheet.
3. In the event of a discrepancy between the Scantron card and what has been noted on the exam booklet, the results on the Scantron are taken as final.

Notice:

1. Short answer questions answered in pencil will not be re-graded, in accordance with the Rules on Academic Misconduct.
2. No marks will be given for illegible and otherwise unreadable, incomprehensible, or unclear answers or writing.
3. You are not allowed to have/use programmable calculators, wallets/purses, cell phones or any such aid under any circumstance unless previously authorized.
4. You are not allowed to leave before 40 minutes or during the last 15 minutes. You may leave the room between 6:10 pm and 6:30 pm.
5. If you need to use the bathroom, notify an invigilator and you will be escorted to the bathroom.

1	2	3	4	5	6	Total
4.0	3.5	4.0	3.0	2.5	3.0	20 marks

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Section 1: Multiple choice questions (20 questions = 20 marks)

1. Two amino acids of the standard 20 contain sulfur atoms. They are:
    A. cysteine and serine
    B. methionine and cysteine*****
    C. methionine and serine
    D. threonine and serine

2. Which of the following amino acids would have the **highest** relative mobility (R_F) in normal thin layer chromatography?
    A. Arginine
    B. Lysine
    C. Methionine*
    D. Asparagine

3. Which of the following amino acids is **least** likely to act as a nucleophile in an enzymatic reaction?
    A. cysteine
    B. serine
    C. histidine
    D. valine*

4. 3-methylhistidine is a post-translationally modified form of histidine with a side chain pKa of 5.8. What is the net charge on 3-methylhistidine side chain at pH 6.5? (The ionization states of 3-methylhistidine are same as that of histidine).
    A. -0.83
    B. -0.17
    C. +0.17*
    D. +0.5

5. How many amino acids are there in one turn of an α-helix?
    A. 1
    B. 2.8
    C. 3.6 *
    D. 4.2

6. What is the net charge on the following peptide at pH 6.5?

    Ala-Lys-Glu-Arg-Gln-Met-His-Thr-Asp-Glu-Gly

    A. -0.5*
    B. -1
    C. +1.5
    D. +0.5

7. When an atom with a lone pair of electrons attracts the hydrogen atom of an -OH or an -NH group, it's acting as a:
   A. Base
   B. Electrophile
   C. Nucleophile
   D. H-bond acceptor*

8. A polypeptide is cleaved into peptides by treatment with the enzyme trypsin, and then the peptides are purified and sequenced. The sequences of the peptides are shown below. Identify the fragment derived from the c-terminus of the original protein.
   A. SGMAIHNK
   B. NSKPMEFAFEEG*
   C. VPR
   D. VMPDLLIEMATLK

9. Mass spectrometry-based sequencing of a peptide results in peaks of the fragments, listed here in Daltons: 156.10111, 285.1437, 414.18629, 561.25471, 698.31362, 769.35073, 866.40349, 967.45117, 1066.51959

   Using the list of masses of amino acids given below, what is the identity of the **N-terminal** amino acid in the peptide?

   Amino acid masses (Da)

G: 57.02147	**R:** 156.10111	**K:** 128.09497	**V:** 99.06842
S: 87.03203	**H:** 137.05891	**D:** 115.02695	**E:** 129.04259
T: 101.04768	**Y:** 163.06333	**N:** 114.04293	**F:** 147.06842
P: 97.05276	**A:** 71.03711		

   A. Thr
   B. Val*
   C. Tyr
   D. Asp

10. In the α-helix, the hydrogen bonds:
    A. are roughly parallel to the axis of the helix. *
    B. are roughly perpendicular to the axis of the helix.
    C. occur mainly between electronegative atoms of the R groups.
    D. occur only near the amino and carboxyl termini of the helix.

11. From step to step, during the multi-step purification of an enzyme, we expect that the **specific activity** of the preparation will:
    A. remain nearly constant
    B. increase *
    C. decrease
    D. increase or decrease depending on the purification methods used.

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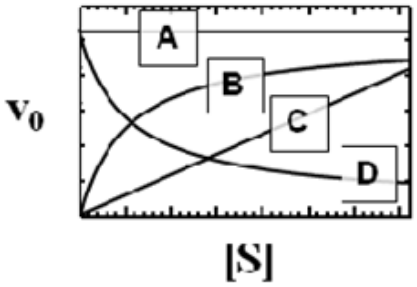
12. By adding SDS (sodium dodecyl sulfate) during the electrophoresis of proteins, it is possible to:
    A. determine a protein's isoelectric point.
    B. determine the amino acid composition of the protein.
    C. preserve a protein's native structure and biological activity.
    D. separate proteins exclusively on the basis of molecular weight.*

13. Two dimensional electrophoresis allows the separation of;
    A. proteins with similar isoelectric points that differ in molecular weight.*
    B. proteins with similar polarity that differ in molecular weight.
    C. proteins with similar charge that differ in molecular weight.
    D. proteins with similar shapes that differ in molecular weight.

14. Why is coupling of the PITC reagent to the N-terminal amino acid of a peptide in the protein sequencing technique developed by Per Edman carried out under basic (alkaline pH) conditions?
    A. High pH unfolds the protein exposing the peptide bonds.
    B. The high concentration of protons destabilizes the peptide carbonyl group.
    C. High pH deprotonates the N-terminal amino group making the N nucleophilic.*
    D. High pH maintains the charge on the side chains so they can be detected.

15. Which of the graphs shown on the right best represents the initial velocity (V_0) of the reaction as a function of substrate concentration?
    A. A
    B. B*
    C. C.
    D. D



16. What key finding did Linus Pauling discover that revolutionized our understanding of protein structure?
    A. The peptide bond has double bond character.*
    B. Upper atmospheric testing of megaton nuclear warheads is bad for proteins.
    C. Quantum calculations explain the hybridization of orbitals.
    D. Peptide bond hydrolysis occurs through differences in electronegativity.

17. The isoelectric point of a protein is:
    A. The pH at which the net charge on the protein is zero.*
    B. The pH at which 50% of the amino acids are deprotonated.
    C. The pH at which 50% of the amino acids are protonated.
    D. The pH at which the protein is denatured.

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18. Which of the following procedures in polypeptide analysis is cyanogen bromide used for?
    A. As a colour reagent to detect amino acids.
    B. To reduce disulfide bonds
    C. In the cyclization step of the Edmen reaction
    D. To cut the polypeptide chain at methionine*

19. Michaelis and Menten assumed that the overall reaction for an enzyme-catalyzed reaction could be written as:

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

    Using this reaction, the rate of breakdown of the enzyme-substrate complex can be described by the expression:
    A. k_1 ([E_t]-[ES])[S]
    B. k_2 [ES]
    C. k_{-1} [ES] + k_2 [ES] *
    D. k_{-1} [ES]

20. In the structure of the α-helix, which pair of groups is hydrogen bonded together?
    A. α-NH of amino acid #1 to α-CO of amino acid #3
    B. α-NH of amino acid #1 to α-CO of amino acid #5
    C. α-CO of amino acid #1 to α-NH of amino acid #4
    D. α-CO of amino acid #1 to α-NH of amino acid #5*

------ MAKE SURE YOUR STUDENT ID NUMBER IS ON THE SCANTRON CARD! ----

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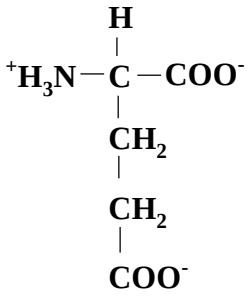
Short answer questions (total marks = 20)

1. Amino acids (3 marks)

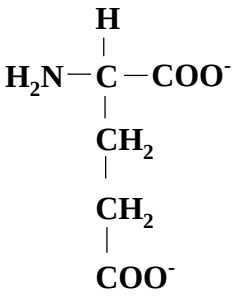
Draw the structure of the amino acid glutamate in its predominant form found at:

i. pH 7.0 (1.5 marks)

ii. pH 11.0 (1.5 marks)



pH 7.0



pH 11.0

2. Enzyme assays and activity (3 marks)

An enzyme assay was performed in a spectrophotometer cuvette at 340 nm. The total assay period was 8 min. At the end of the 8 minutes, the absorbance of the assay mixture increased by 0.46. Based on this value, the increase in concentration of the product of the reaction was calculated to be 7.41 x 10^{-4} Mol L^{-1}.

i. If the volume of the reaction mixture was 5 mL and the amount of enzyme added was 12 µg, calculate the **specific activity** of the above enzyme. Show all your work for full marks. (2 marks)

Rate of enzyme reaction = 7.41 x 10^{-4} Mol L^{-1}/8 min (0.5 marks)
= 9.26 x 10^{-5} Mol L^{-1}.Min^{-1}

Enzyme Activity = (9.26 x 10^{-5} Mol.L^{-1}.Min^{-1}) x (5 x 10^{-3} L) (0.5 marks)
= 4.63 x 10^{-7} Moles. Min^{-1}
= 0.463 µMoles.min^{-1}

Specific Activity = 0.463 µMoles.min^{-1}/12µg (1 mark)
= 3.85 x 10^{-2} µMoles.min^{-1}.$µg^{-1}$

ii. If the molar mass of the enzyme is 35 kDa, calculate the molar activity of the reaction. (1 mark)

Molar activity = SA x Molar mass
= 3.85 x 10^{-2} µMoles.min^{-1}.$µg^{-1}$ x 35000 ug/umol/60 s
=22.5 s^{-1}

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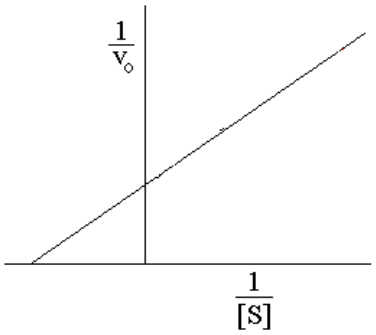
3. Enzyme kinetics (3 marks)

i. The figure shows a linear plot of kinetic data (a Lineweaver-Burke plot) for an enzyme reaction. If the x-intercept is -3.3 x 10^3 L mol^{-1}, the y-intercept is 6.25 x 10^4 L mol^{-1} min and the slope is 18.75 min, calculate the K_M for the enzyme. (1 mark)



x-intercept = $-1/K_M$

$-1/K_M = -3.3 \times 10^3$ L.Mol^{-1}

$K_M = -1/-3.3 \times 10^3$

$= 3.03 \times 10^{-4}$ Mol.L^{-1} (M) (1 mark)

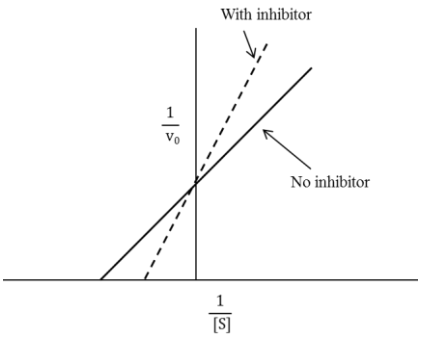
ii. For the above enzyme, what initial reaction velocity (v_0) would be observed if the substrate concentration is 0.5 x K_M and V_{max} is 1.6 x 10^{-5} Mol.L^{-1}.min^{-1} (1 mark)

$$\frac{V_0}{V_{max}} = \frac{0.5\ K_M}{K_M + 0.5\ K_M}$$

$$= \frac{0.5}{1.5} \times 1.6 \times 10^{-5}\ \text{Mol.L}^{-1}.\text{min}^{-1}$$

$$= 5.33 \times 10^{-6}\ \text{Mol.L}^{-1}.\text{min}^{-1}$$

iii. The experiment in (i) above, was carried out again in the presence of an inhibitor. The Lineweaver-Burke plot in the presence of the inhibitor had the same Y-intercept but a lower X-intercept. Based on this information, determine the type of inhibition caused by the inhibitor. (1 mark)



Competitive inhibition

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4. Protein secondary and tertiary structure (4 marks)

Shown below is the amino acid sequence of a segment of a protein. It can be divided into three regions of secondary structure, separated by two short clusters of secondary structure breakers as shown by the boxed amino acids.

Ile-Thr-Trp-Gln-Val-[Pro-Lys-Ser-Asp]-Trp-Thr-Met-Glu-Tyr-His-Ile-Thr-[Gly-Pro-Phe-Asn]-Arg-Val-Thr-Leu-Thr-Ile
NP-P-MNP-P- NP MNP- P- NP- P- MNP-P- NP-P P- NP- P- NP- P- NP

i. Based on the amino acids present in each of the segments between the breaker clusters, determine the **secondary structure** of **each segment.** (1.5 marks)

Beta-beta-beta

ii. Which family of protein **tertiary structures** is this sequence likely to be found in? (1 mark).

Beta Barrel (1)

iii. Briefly explain how the distribution of polar and non-polar amino acids in this oligopeptide determines the way it folds into this tertiary structure. (1.5 mark)

- Amino acids in each segment alternates polar and non-polar (0.5)
- This will lead one side of the sheet to be non-polar and the other to be polar (0.5).
- The sheet will then fold with the non-polar side facing the interior and the polar side facing the exterior forming a beta barrel. (0.5)

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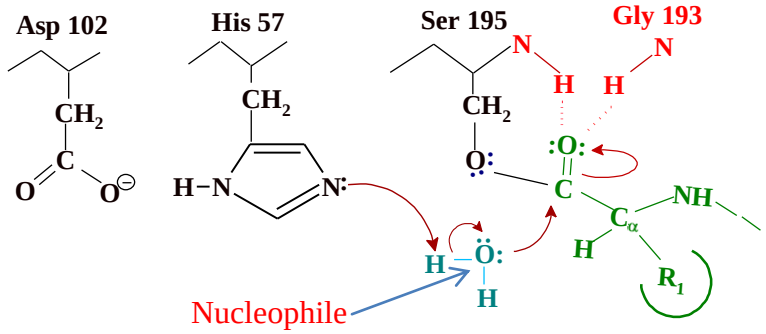
5. Chymotrypsin mechanism (4 marks)

Shown below is the start of the second step of the chymotrypsin mechanism. The N-terminus of the protein undergoing hydrolysis is covalently bound to the enzyme to form the acyl-enzyme intermediate and a molecule of water has entered the catalytic site.



i. Label the atom that acts as the nucleophile in this step. (1 mark)

ii. Indicate **the movement of electrons** that occurs in this step leading to the formation of the second transition state. (2 marks)

iii. What form of enzyme catalysis is His-57 performing in this step (0.5 marks)? How does this help the reaction mechanism? (0.5 marks)

General base catalysis

Makes O a better nucleophile

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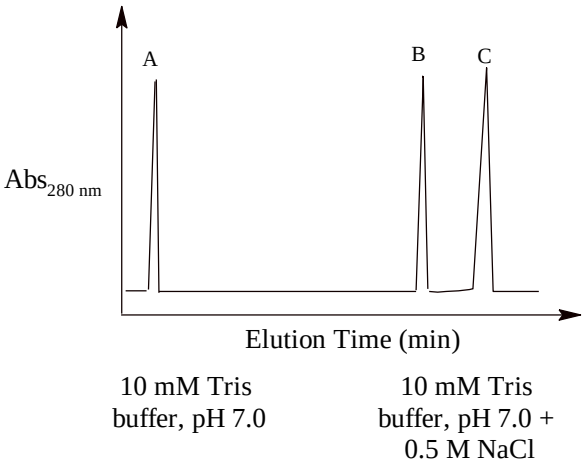
6. Chromatography (3 marks)

i. A mixture of peptides is separated using **anion** exchange chromatography (right). The peptides were eluted with the solutions indicated below the chromatogram. Identify which of the three peptides shown below corresponds to peaks A, B and C. (1.5 marks)



1. Thr-Asp-Val-Glu-Ile-Lys-Cys-Gln = -1 (B)
2. Met-Cys-Lys-Asn-Gly-Phe-Lys-Pro-Leu-Arg-Tyr-Asn = +3 (A)
3. Glu-Ser-Ala-Phe-Asp = -2 (C)

B. If the above three peptides were run through a gel filtration column, indicate the order of their elution. (Identify each protein by the number assigned to it, 1, 2 or 3) (1.5 marks)

First: __2_____

Second: __1___

Third: ____3__

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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		

α-amino group = 9.6
α-carboxylate group = 2.4

Average N-terminal amino group has pKa = 9.5
Average C-terminal carboxylic acid group has pKa = 2.5

Nothing below will be marked. Use for rough work.