Name _____ **Student number** _____

University of Guelph
Department of Molecular and Cellular Biology

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Enter this code in **question 21** on the scantron card

F2011 BIOC*2580 Introductory Biochemistry

Midterm
Sat Oct 22 2011, 10:00–11:15 am RozH 101/103/104

This exam determines 25% of the final course grade

Instructors: Drs. Frances Sharom and 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, palm pilots, blackberries, etc. 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 10:40 am and 11:00 am.
5. If you need to use the bathroom, notify an invigilator and you will be escorted to the bathroom.

2.1	2.2	2.3	2.4	2.5	2.6	Total
3 marks	2.5 marks	3.5 marks	2.5 marks	2 marks	6.5 marks	20 marks

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Section 1: Multiple choice questions (20 questions = 30 marks)

You should spend about 45 minutes on this section

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

   Glu-Ala-Arg-Met-Gln-Asp-His-Ser

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

2. Which of the following amino acids will bind to anion exchange resin at pH 7.0?

   A. Asp
   B. Asn
   C. Lys
   D. Leu

3. In a mixture of the four proteins listed below, which should elute **second** in gel filtration (size-exclusion) chromatography?

   A. ovalbumin, M_r = 45 kDa
   B. immunoglobulin, M_r = 145 kDa
   C. ribonuclease A, M_r = 13.7 kDa
   D. serum albumin, M_r = 68.5 kDa

4. A polypeptide is cleaved into peptides by treatment with trypsin and cyanogen bromide, and then the peptides are purified and sequenced. The sequences of the peptides are shown below.

Trypsin peptides	Cyanogen bromide peptides
T-1 GSMDPVALMTLR	C-1 TLRNFEEGSKFENYA
T-2 ITGLAIHQK	C-2 ITGLAIHQKELIM
T-3 NFEEGSK	C-3 VPKGSM
T-4 ELIMVPK	C-4 DPVALM
T-5 FENYA	

Based on sequences of the overlapping peptides generated by treatment with trypsin and cyanogen bromide (shown above), which of the following peptides represents the **N-terminus** of the original polypeptide?

   A. T-3
   B. C-1
   C. C-2
   D. T-5

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5. Which of the following is **NOT** true about SDS-polyacrylamide gel electrophoresis of proteins?

   A. It separates proteins that differ in molecular weight
   B. SDS denatures the proteins
   C. An electric field is used to move the proteins through the gel
   D. Larger proteins move through the gel faster than smaller proteins

6. Ashley the biochemist completed mass spectrometry-based sequencing of a peptide from her protein and then accidently shredded the peptide sequencing results! She was able to find the mass values for the peaks of the fragments, listed here in Daltons: 156.10111, 271.12806, 372.17574, 486.21867, 649.282, 748.35042, 849.3981, 978.44069.

   Using the list of masses of amino acids given below, what is the identity of the **third** amino acid in the peptide when the sequence is written N- to C-terminus?

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

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

7. Which of the following groupings correctly shows intrachain hydrogen bonding (…) in a protein α-helix?

   A. –N-H … H-N-
   B. –C=O … H-C-
   C. –C=O … H-N-
   D. –N-H … H-R-

8. Which level of protein structure describes the sequence of amino acids in a polypeptide?

   A. Quaternary structure
   B. Secondary structure
   C. Primary structure
   D. Tertiary structure

9. Which of the following amino acids would most likely be located on the surface of a protein?

   A. Arg
   B. Trp
   C. Ala
   D. Leu

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10. Which of the following interactions contributes most to the tertiary structure of proteins?

    A. Hydrophobic effect
    B. Hydrogen bonding
    C. Van der Waals interactions
    D. Electrostatic interactions between charged side chains

11. To which family of tertiary structures does the following sequence most likely belong?

    Val-Thr-Tyr-Thr-Ile-Asp-Ile-Thr-Trp

    A. α/β barrel
    B. four-helix bundle
    C. β-barrel
    D. Greek key

12. The structure on the right belongs to which family of protein tertiary structures?

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

13. Which of the following experiments provided the first evidence that the amino acid sequence of a polypeptide chain contains all the information required to fold the chain into its native, three-dimensional structure?

    A. Addition of mercaptoethanol causes ribonuclease to regain catalytic activity
    B. When ribonuclease is treated with urea, it loses its catalytic activity
    C. When renatured ribonuclease is allowed to denature, it regains its catalytic activity
    D. When denatured ribonuclease is allowed to renature, it regains its catalytic activity

14. Atoms do **NOT** employ a lone pair of electrons as a:

    A. General base
    B. General acid
    C. Nucleophile
    D. Hydrogen bond acceptor

15. Which of the following makes a major contribution to the increase in reaction rate by enzymes?

    A. Altering the pH of the surroundings by uptake or release of H^+
    B. Reducing the collision frequency between the reactants
    C. Destabilizing the transition state
    D. Holding the reactants close together long enough for the reaction to proceed

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16. Indicate which peptide bonds (labelled 1-14) in the peptide below are cut by chymotrypsin.

```
   1   2   3   4   5   6   7   8   9   10  11  12  13  14
Leu-Arg-Asn-Pro-Phe-Val-Met-Lys-Trp-His-Tyr-Ala-Tyr-Pro-Cys
```

A. 5, 9 and 11
B. 2 and 8
C. 5, 9, 11 and 13
D. 9 and 11

17. What is the benefit of measuring the **initial rate** of reaction, v_0, at the beginning of an enzyme-catalyzed reaction?

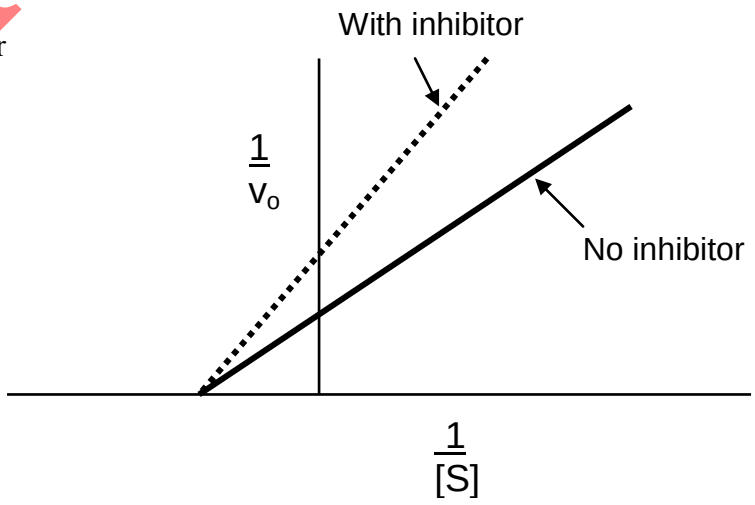
A. [ES] can only be measured accurately at t=0
B. At t=0, [P] = 0, so the reverse reaction can be ignored
C. $v_0 = V_{max}$ at t=0
D. K_M is at a minimum at t=0

18. You have engineered and purified a mutant version of an enzyme in which a Ser residue is replaced with Ala. When you carry out kinetic analysis on the mutant, you find that its V_{max} is the same as the wild-type enzyme, but the K_M is 40-fold higher. What conclusions can you draw from these observations?

A. The Ser residue plays a critical role in catalysis
B. The Ser residue is important for recognizing and binding the substrate
C. The enzyme has a higher turnover number when Ala replaces Ser
D. The enzyme has a lower turnover number when Ala replaces Ser

19. Shown below is a Lineweaver-Burk plot of kinetic data for an enzyme in the presence and absence of an inhibitor. What kind of inhibitor is it?

A. Non-competitive inhibitor
B. Competitive inhibitor
C. Mixed inhibitor
D. Inactivator



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20. At what percentage of its V_{max} does an enzyme function when the substrate concentration is 20% of the K_M?

    A. 83.3%
    B. 20%
    C. 16.6%
    D. 10%

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If you haven't already

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

Use the rest of this page for rough work.

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Short answer questions (total marks = 20)

You should spend about 30 minutes on this section

2.1 Peptide structure (3 marks)

Draw the complete structure of the simple dipeptide Glu-Phe, showing the ionizable groups in their predominant forms at pH 7.0.

2.2 Enzyme assays and activity (2.5 marks)

a) You are assaying a dehydrogenase enzyme that uses NAD^+, which is converted to NADH. At the end of the 5 minute assay period, the increase in absorbance of the reaction mixture at 340 nm in a 0.5 cm cuvette is 0.62. What is the concentration of the NADH product, given that the extinction coefficient for NADH is 6200 L mol^{-1} cm^{-1} at 340 nm? Show all your work. **(1 mark)**

b) Given that the cuvette in which the reaction is carried out has a volume of 2.5 mL, what is the activity of the enzyme sample in part a), in μmol min^{-1}?
Show all your work. **(1.5 marks)**

2.3 Mechanism of chymotrypsin (3.5 marks total)

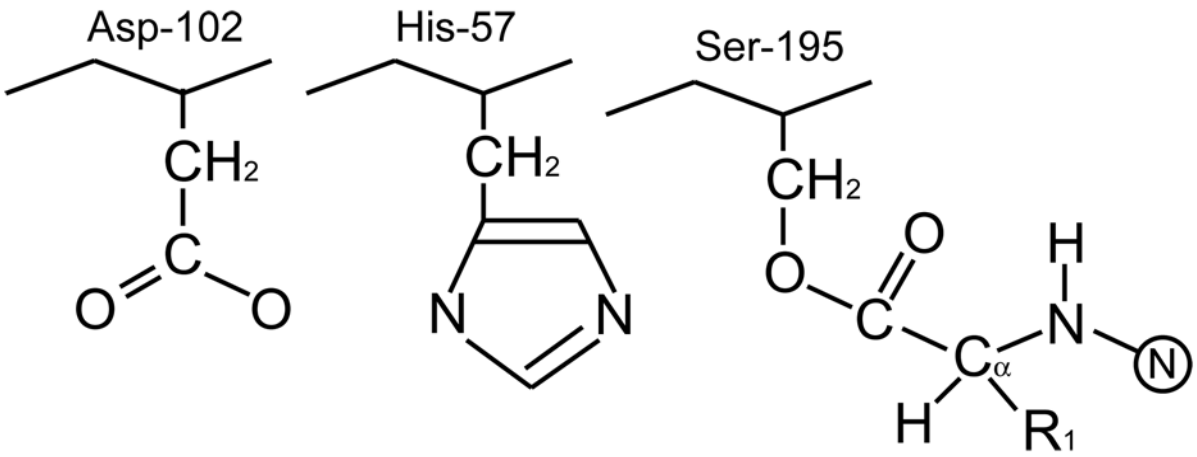
Chymotrypsin increases the rate of hydrolysis of peptide bonds by breaking the reaction mechanism into two lower energy steps. At the end of the first step, the peptide bond is broken, releasing the C-terminal peptide, while the N-terminal peptide remains covalently bound to the chymotrypsin enzyme in the acyl-enzyme intermediate.

Shown below is a representation of the active site in the acyl-enzyme intermediate of chymotrypsin. Perform the following on this diagram:

1. Complete the structures of the amino acids participating in the catalytic mechanism by indicating any formal charges or hydrogen atoms. **(0.5 mark)**

2. Draw a **water** molecule where it would located before the formation of the second transition state in the chymotrypsin catalytic mechanism. **(1 mark)**

3. Indicate **the movement of electrons** that occurs leading to the formation of the second transition state in the chymotrypsin catalytic mechanism. (**2 marks**)



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2.4 Enzyme mechanisms: chymotrypsin (2.5 marks total)

The chymotrypsin enzyme vastly increases the rate of peptide bond breakage by water. **Match** the general examples of enzyme modes of action and binding forces to the specific examples from the chymotrypsin mechanism. Write the letter associated with the general examples in the space beside the specific example. Not all of the general examples are used.

Example:

___J__ The binding of a large amino acid in the binding pocket of chymotrypsin positions the peptide bond to be broken close to the catalytic unit.

_____ The binding pocket of chymotrypsin is the right size to fit a large amino acid.

_____ Chymotrypsin breaks the peptide hydrolysis reaction into two easier steps.

_____ His-57 donates or accepts protons during catalysis.

_____ The binding pocket of chymotrypsin is lined with non-polar amino acids.

_____ The oxyanion hole binds to the tetrahedral carboxyanion.

A. General acid/base catalysis
B. Nucleophilic catalysis
C. Lowering the energy of activation
D. Complementary to the transition state
E. Hydrogen bonds
F. van der Waals forces
G. Ionic interactions
H. Hydrophobic effect
I. Electrophilic catalysis
J. Proximity and orientation

2.5 Enzyme kinetics (2 marks)

Given the enzymatic reaction: $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$

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

2.6 Protein structure (6.5 marks total)

a) What **TWO** structural properties make certain amino acids prefer β-sheet structure? **(2 marks)**

i)

ii)

b) Draw a box around each cluster of secondary structure breakers in the peptide shown below. **(1 mark)**

IWCTYQVAVSKPGALQMIVEHARLDNTPWVFNCI

Based on the amino acids present in the segments between the breakers, what do you predict the **secondary structure** of each segment to be, and why? **(2 marks)**

c) Write one sentence explaining how the following reagents are used in the study and analysis of proteins. **(1.5 marks)**

ninhydrin:

urea:

fluorodinitrobenzene:

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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		

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.