

Name _____ Student number _____

University of Guelph
Department of Molecular and Cellular Biology

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

CONTROL CODE B Enter this code in question 21 on the scantron card

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

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?

Asp-Ala-Lys-Cys-Asn-Arg-His-Thr

- A. +0.5
- B. +1
- C. +1.5*
- D. +2

2. Which of the following amino acids will bind to cation 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 **third** 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 FENYA	C-1 DPVALM
T-2 ELIMVPK	C-2 VPKGSM
T-3 NFEEGSK	C-3 ITGLAIHQKELIM
T-4 ITGLAIHQK	C-4 TLRNFEEGSKFENYA
T-5 GSMDPVALMTLR	

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-1
- B. T-4*
- C. C-2
- D. C-4

5. Which of the following is **NOT** true about SDS-polyacrylamide gel electrophoresis of proteins?
- A. Larger proteins move through the gel faster than smaller proteins*
 - B. It separates proteins that differ in molecular weight
 - C. An electric field is used to move the proteins through the gel
 - D. SDS denatures the proteins

6. Ashley the biochemist completed mass spectrometry-based sequencing of a peptide from her protein and then accidentally 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 *fourth* 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. Tyr*
 - B. Asn
 - C. Thr
 - D. Val
7. Which of the following groupings correctly shows intrachain hydrogen bonding (...) in a protein α -helix?
- A. $-N-H \dots H-N-$
 - B. $-N-H \dots H-R-$
 - C. $-C=O \dots H-C-$
 - D. $-C=O \dots H-N-^*$
8. Which level of protein structure describes all aspects of the three-dimensional folding of a polypeptide?
- A. Primary structure
 - B. Secondary structure
 - C. Tertiary structure*
 - D. Quaternary structure
9. Which of the following amino acids would most likely be located in the interior of a protein?
- A. Arg
 - B. Thr
 - C. Lys

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D. Leu*

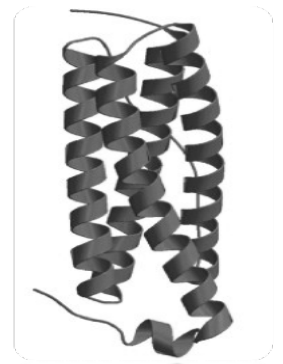
10. Which of the following interactions contributes most to the tertiary structure of proteins?

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

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

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

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



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

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

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

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

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

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

D. Reducing the collision frequency between the reactants

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

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

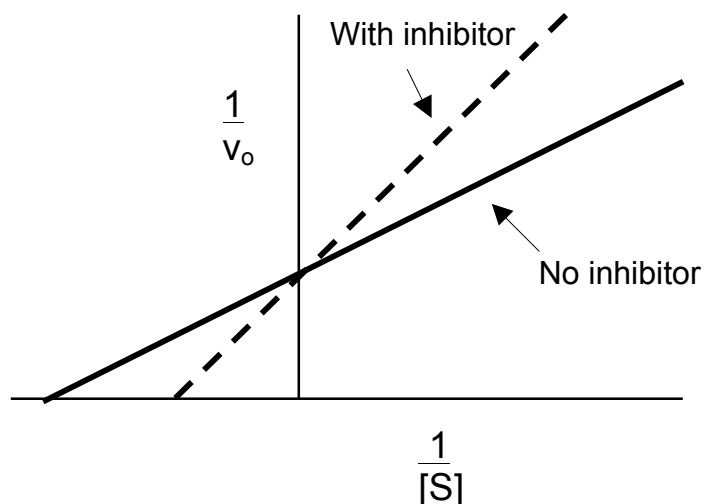
- A. K_M is at a minimum at $t=0$
- B. $v_0 = V_{max}$ at $t=0$
- C. $[ES]$ can only be measured accurately at $t=0$
- D. At $t=0$, $[P] = 0$, so the reverse reaction can be ignored*

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

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. 10%
- B. 16.6%*
- C. 20%
- D. 83.3%

21.

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if you haven't already

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

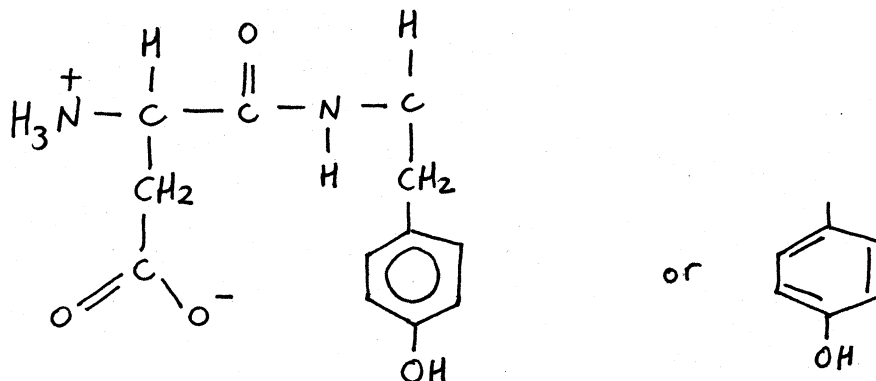
Use the rest of this page for rough work.

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 Asp-Tyr, 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)

$$A = \epsilon l c, \text{ so } c = A / \epsilon l$$

$$c = 0.62 / (6200 \times 0.5) \text{ mol. L}^{-1} \text{ cm. cm}^{-1} = 2 \times 10^{-4} \text{ M or mol.L}^{-1}$$

- 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 $\mu\text{mol min}^{-1}$? Show all your work. (1.5 marks)

$$\text{Rate of enzyme reaction} = 2 \times 10^{-4} \text{ mol L}^{-1} / 5 \text{ min} = 4 \times 10^{-5} \text{ mol L}^{-1} \text{ min}^{-1}$$

$$\begin{aligned} \text{Enzyme activity} &= \text{rate} \times \text{vol of enzyme} = 4 \times 10^{-5} \text{ mol L}^{-1} \text{ min}^{-1} \times 2.5 \times 10^{-3} \text{ L} \\ &= 1 \times 10^{-7} \text{ mol min}^{-1} \end{aligned}$$

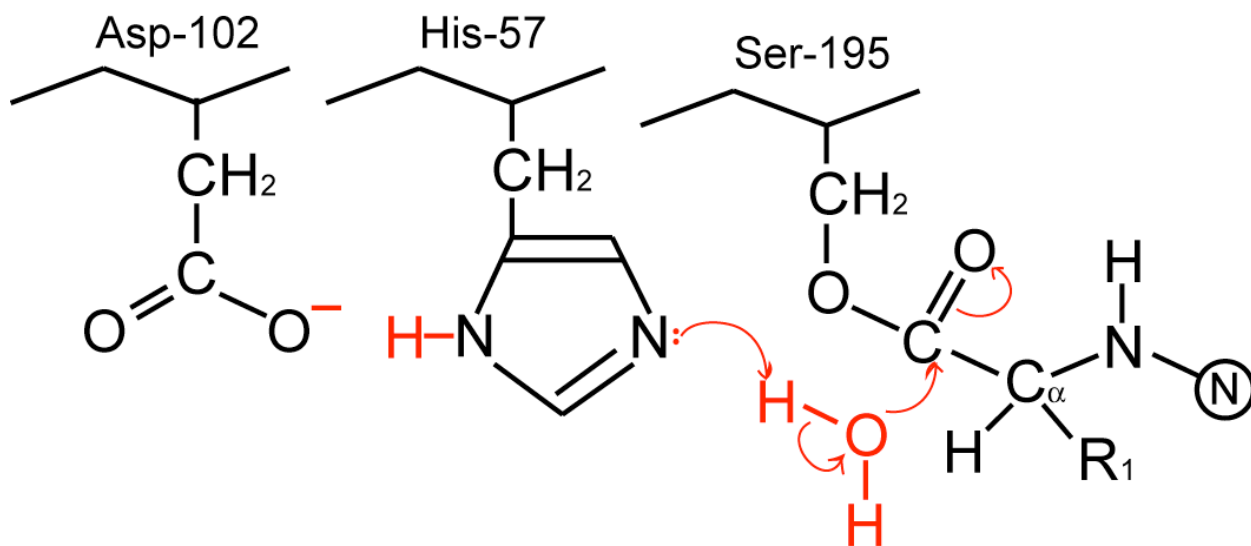
$$\text{Convert units: } 1 \times 10^{-7} \text{ mol min}^{-1} \times 10^6 \mu\text{mol mol}^{-1} = 0.1 \mu\text{mol min}^{-1}$$

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 be 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)**



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.

___A___ His-57 donates or accepts protons during catalysis.

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

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

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

___D___ The oxyanion hole binds to the tetrahedral carboxyanion.

A. General acid/base catalysis

F. van der Waals forces

B. Nucleophilic catalysis

G. Ionic interactions

C. Lowering the energy of activation

H. Hydrophobic effect

D. Complementary to the transition state

I. Electrophilic catalysis

E. Hydrogen bonds

J. Proximity and orientation

2.5 Enzyme kinetics (2 marks)

Given the enzymatic reaction:



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

rate of breakdown of ES = rate of formation of ES

rate of breakdown of ES = $k_2[ES] + k_{-1}[ES] = (k_2 + k_{-1})[ES]$

rate of formation of ES = $k_1[E][S]$

Therefore,

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

The Michaelis constant, K_M , is an amalgamation of these rate constants:

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

2.6 Protein structure (6.5 marks total)

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

i) **A branch on the β -carbon of the amino acid**

ii) **A large or bulky amino acid**

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

ICNFVW**PTND**LRAHEVIMQLA**GPKS**VAVQYTCWI
 $\beta \beta \alpha \beta \beta \beta$ $\alpha \alpha \alpha \alpha \alpha \beta \beta \alpha \alpha \alpha$ $\beta \alpha \beta \alpha \beta \beta \beta \beta$

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)**

The two clusters of breakers separate the peptide into 3 segments. The first and third segments are composed almost predominantly of β -formers, while the second segment is mainly α -formers. The “local majority rules” in terms of deciding secondary structure, **so the peptide will have the structure β - α - β , separated by the two breaker sections.**

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

ninhydrin:

Ninhydrin reacts with amino groups to give a purple colour, and is used to detect and measure the quantity of amino acids on (for example) TLC plates.

urea:

Concentrated solutions of urea weaken the hydrophobic effect, causing proteins to unfold from their native tertiary structure and change to the denatured state.

fluorodinitrobenzene:

Fluorodinitrobenzene reacts with the amino group of the N-terminal amino acid of a peptide/protein, which can then be identified after hydrolysis based on the yellow colour of the labelled amino acid.

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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.