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University of Guelph
Department of Molecular and Cellular Biology

F'15 BIOC*2580 Introductory Biochemistry

Midterm
Sat. Oct. 26 2015, 11:30 am – 12:45 pm RozH 101/104

Instructor: Dr. Steffen Graether

Total marks: 40 Total time: 75 minutes Total pages in this midterm: 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. Answer the multiple choice questions in section 1 on the Scantron sheet provided. Answer section 2 in the space provided on this exam.
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 sheet and erase cleanly if needed. Do NOT use white-out or ink on the Scantron sheet.
2. Fill out your **name and ID number** on the Scantron sheet.
3. In the event of a discrepancy between the Scantron sheet and what has been noted on the exam booklet, the results on the Scantron are taken as final

Note:

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, etc. or similar aid under any circumstances, unless previously authorized.
4. You are not allowed to leave before 30 minutes or during the last 15 minutes. You may leave the room between noon and 12:30 pm.
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	Total
4 marks	4 marks	4 marks	4 marks	4 marks	20 marks

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

Correct answers are marked with *

1. What is the net charge on the following peptide at pH 4.0?

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

   A. –3.0
   B. –2.5
   C. +2.5 *
   D. +3.0

2. Which of the following amino acids will bind to an anion exchange resin at pH 7.5?

   A. Gln
   B. Glu *
   C. Gly
   D. All of the above

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

   A. ovalbumin, M_r = 45 kDa
   B. carbonic anhydrase, M_r = 30 kDa
   C. lysozyme, M_r = 14 kDa
   D. titan, M_r = 1000 kDa *

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

Elastase peptides	Cyanogen bromide peptides
E-1 EPFSVLMNERDKA	C-1 NERDKAQSVINM
E-2 STVDMYA	C-2 YAEPFSVLM
E-3 ETPFDSSQ	C-3 GKLLRAETPFDSSQ
E-4 QSVINMG	C-4 STVDM
E-5 KLLRA	

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

   A. E-3 *
   B. C-1
   C. C-2
   D. E-4

5. In the structure of a polypeptide 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 #4
   C. α-CO of amino acid #1 to α-NH of amino acid #5 *
   D. A) and/or B), depending on where you are in the helix

6. A biochem student completed mass spectrometry-based sequencing of a peptide of her protein and then accidently shredded the peptide sequencing results (too much fun at the SLG session the night before). She was able to find the mass values for the peaks of the fragments, listed here in Daltons (Da): 128.09; 227.16; 328.21; 443.23; 629.31; 700.35; 771.38.

Using the list of masses of amino acid residues (below), what is the identity of the **third** amino acid residue in the peptide when the sequence is written in the standard way (N- to C-terminus)?

Amino acid residue masses (Da)

G: 57.02147	**R:** 114.04293	**K:** 128.09497	**V:** 99.06842
S: 87.03203	**W:** 186.07932	**D:** 115.02695	**A:** 71.03712
T: 101.04768	**Y:** 163.06333	**N:** 114.04293	**F:** 147.06842

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

7. Which of the following is **TRUE** about SDS-polyacrylamide gel electrophoresis of proteins?

   A. It separates proteins that are the same in molecular weight
   B. SDS keeps the proteins native
   C. A pH gradient is used to move the proteins through the gel
   D. Smaller proteins move through the gel faster than larger proteins *

8. Which of the following amino acids would most likely be located in the interior of a protein?

   A. Asp
   B. Leu *
   C. Lys
   D. Ser

9. Which of the following interactions contributes **most** to the tertiary structure of a globular protein?

   A. Hydrophobic effect *
   B. Hydrogen bonding
   C. Polar interactions between polar side chains
   D. Electrostatic interactions between charged side chains

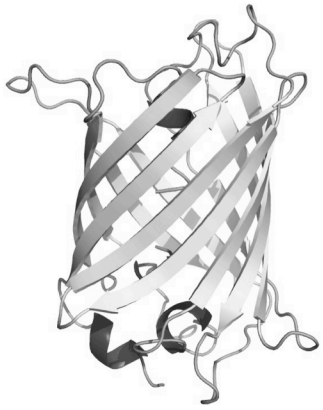
10. To which family of secondary structures does the following sequence most likely belong?

AVRFAIQEMK

A. β-strand
B. α-helix *
C. α-barrel
D. α-sandwich

11. The structure below belongs to which family of protein tertiary structures?

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



12. Which of the following experimental findings 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 2-mercaptoethanol causes ribonuclease to regain catalytic activity
B. When denatured ribonuclease is allowed to renature, it regains its catalytic activity *
C. When ribonuclease is treated with urea, it loses its catalytic activity
D. When ribonuclease is treated with 2-mercaptoethanol, it loses its catalytic activity

13. You have a 4 μg enzyme sample with an activity of 15 μmol min^{-1}. If the molar mass of the enzyme is 60 kDa, how many molecules of substrate does one enzyme molecule convert to product per second?

A. 3.75
B. 225
C. 3,750 *
D. 22,500

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

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

A. 2 and 5 *
B. 2, 5, and 8
C. 6 and 11
D. 6, 11 and 13

15. Which of the following statements is **TRUE**?

A. A general base donates a proton
B. A general acid accepts a proton
C. Gain of a proton in a small space has the same effect as a nucleophile
D. Proton exchange occurs at the site of reaction *

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

A. Holding the reactants in an optimal orientation *
B. Altering the pH of the buffer by uptake or release of H^+
C. Keeping the reactants apart before they are ready to react
D. Destabilizing the transition state

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 be measured accurately at t = 0
B. At t = 0, $v_0 = ½ V_{max}$
C. At t = 0, [P] = 0, so the reverse catalytic reaction can be ignored *
D. K_M reaches a maximum at t = 0

18. You have engineered and purified a mutant version of an enzyme in which a Thr residue is replaced with Ser. When you carry out kinetic analysis on the mutant, you find that its K_M is the same as the wild-type, but the V_{max} is 50-fold lower. What conclusions can you draw from these observations?

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

19. Which of the following statements about v_{max} is **FALSE**?

    A. It is the velocity of an enzymatic reaction when the enzyme is saturated with substrate
    B. It cannot exceed twice the K_M value of an enzymatic reaction *
    C. It is dependent on the enzyme concentration
    D. It is the upper limit for an enzyme rate

20. At what percentage of its V_{max} does an enzyme function when the substrate concentration is 35% of the K_M?

    A. 26% *
    B. 35%
    C. 65%
    D. 74%

Nothing below this line will be marked.

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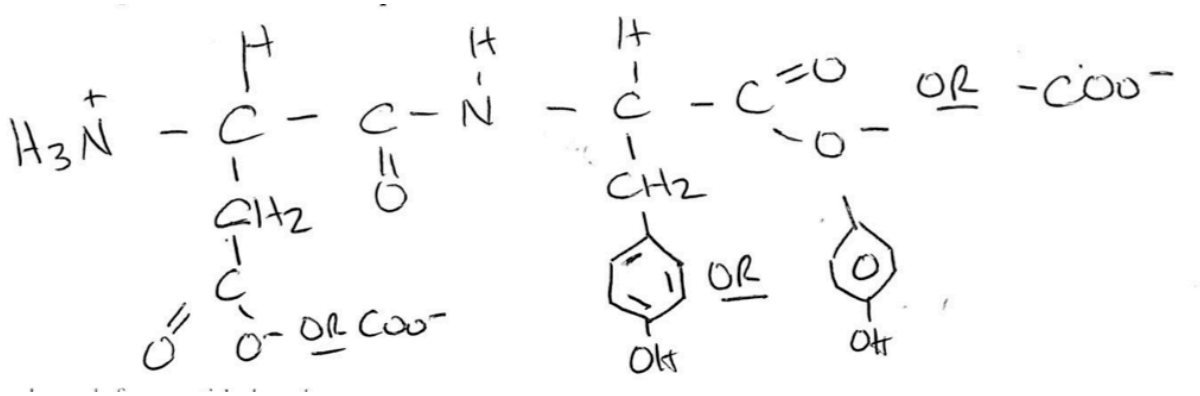
Short answer questions (Total marks = 20)

2.1 Peptide structure (4 marks)

Draw the complete structure (all atoms) of the dipeptide Asp-Tyr, showing the ionizable groups in their predominant forms at pH 7.0.



1 mark for peptide bond
1 mark each for correct side chain and backbone
1 mark for correct charges
(2 marks max for correctly drawing 2 amino acids separately)

2.2 Enzyme assays and activity (4 marks total)

a) You are assaying a dehydrogenase enzyme that uses FAD, which is converted to FADH. At the end of the 5 minute assay period, the increase in absorbance at 450 nm in a 1 cm cuvette is 0.150. Assuming you are starting with zero FAD, what is the concentration of the FAD product in μM, given that the extinction coefficient for FAD is 11300 L mol^{-1} cm^{-1} at 450 nm? Show all your work. **(2 marks)**

$A = \varepsilon l c$, so $c = A/ \varepsilon l$ (1 mark)
$c = 0.150/(11300 \times 1.0)$ mol L^{-1} cm/cm × (10^6 μmol/mol) (to convert the units correctly)
c = 13.3 μM (1 mark)
-1/2 for wrong units
-1/2 for correct value with incorrect exponent

b) Given that the cuvette in which the reaction is carried out has a volume of 1.75 mL, what is the activity of the enzyme sample in part a), in μmol min^{-1}? Show all your work. **(2 marks)**

Rate of reaction= change in concentration of S or P per unit time = 13.3 μM/5 min
= 2.66 μM min^{-1}

Rate = 2.66 μmol L^{-1} min^{-1} (1/2 mark)

Activity = rate × volume = 2.66 μmol L^{-1} min^{-1} × 1.75 mL × ($L/10^3$ mL)(to convert the units correctly) (1/2 mark for some form of this eqn)
Activity = 4.66 x 10^{-3} μmol min^{-1} (1 mark)

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2.3 Net charge of a peptide (4 marks total)

Given the pKa values attached to the end of the midterm, calculate the net charge of the polypeptide at pH 12.8. Show all of your calculations for the partial charges, and indicate the charge on every functional group, even if it is neutral.

Asp-Gly-Asn-Pro-Arg-Arg-Val

$$pH = pKa + \log \frac{[DEPROT]}{[PROT]}$$

$$12.8 = 12.5 + \log \frac{[NH]}{[NH_2^+]}$$

$$\frac{[NH]}{[NH_2^+]} = 10^{0.3} = 1.995 \quad (\text{MAY ROUND TO } 2)$$

$$\frac{\alpha}{1-\alpha} = 1.995 \qquad \alpha = \frac{1.995}{2.995} = 0.666$$

$$\therefore \text{CHARGE} = 1 - \alpha = 0.334 \cdot (+1)$$

$$= +0.334$$

$$\text{NET CHARGE} = -1 + 2(0.334) - 1$$

$$= -1.33$$

Correct charges (not including Arg): 1 mark
Correct alpha: 1 mark
Correct charge (Arg): 1 mark
Correct net charge: 1 mark, only ½ mark if added correctly but based on incorrect whole charges

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2.4 Enzyme mechanisms (4 marks total)

The enzyme chymotrypsin 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:

__**A**__ The binding of a large amino acid in the binding pocket of chymotrypsin positions the peptide bond to be broken close to the catalytic triad.

__**I**___ The binding pocket of chymotrypsin is lined with leucine and tryptophan residues.

__**G**__ Chymotrypsin breaks the peptide hydrolysis reaction into two easier steps.

__**J**__ His-57 donates or accepts protons during catalysis.

__**D**__ The N-terminal end of the peptide substrate binds in a groove on the enzyme surface

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

2.5 Protein secondary structure (4 marks <u>total</u>)

a) Draw a box around the two clusters of secondary structure breakers in the peptide shown below. **(1 mark)**

MGLITMEYQPTTPTCAWFVITCGAASWQTQVYC
β β β β β β β β β β β β β

½ mark for each box – must include all 4 amino acids

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

The first segment is mainly α-formers (**0.5 marks**), while the second and third segments are composed almost predominantly of β-formers (**0.5 marks × 2**). Some form of the "local majority rules" concept in terms of deciding secondary structure (**0.5 marks**).

b) Give an example of an amino acid that prefers to form a β-strand. Explain why that amino acid prefers to form a β-strand. **(1 mark)**

One of Trp (W), Tyr (Y), Phe (F), Val (V), Ile (I), Thr (T) or Cys (C) (1/2 mark)

One of the following answers (1/2 mark):
For any of the above: bulky shape *or* awkward shape
For Trp, Tyr, Phe: they are big/have big side chains
For Val, Ile, Thr: have a branch on the β-C
For Cys: has a large S atom

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pKa values chart:

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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 only.