Name ______________________ **ID number** ________________

Section 1: Multiple choice questions (25 questions = 25 marks)

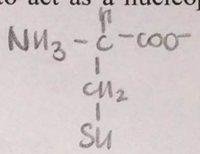
1. Which of the following amino acids would most likely be found on the **exterior** of a protein?
   - ~~A.~~ valine
   - ~~B.~~ isoleucine
   - ~~C.~~ tryptophan
   - [✓ D.] glutamate

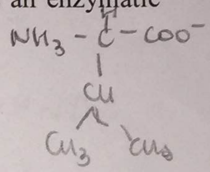
Polar

2. Which of the following amino acids would have the **highest** relative mobility (R_F) in normal thin layer chromatography?
   - ~~A.~~ arginine
   - ~~B.~~ aspartate
   - [✓ C.] methionine
   - ~~D.~~ asparagine

↑ R_f non-polar AA

3. Which of the following amino acids is **least** likely to act as a nucleophile in an enzymatic reaction?
   - ~~A.~~ cysteine
   - ~~B.~~ serine → catalytic triad
   - [✓ C.] valine
   - ~~D.~~ histidine → Catalytic triad





4. Edman's method for determining amino acid sequence involves which of the following two distinct steps?
   - ~~A.~~ Coupling to phenylisothiocyanate (PITC) in mild base followed by hydrolysis in acid.
   - ~~B.~~ Coupling to phenylisothiocyanate (PITC) in anhydrous acid followed by cyclization in mild base.
   - [✓ C.] Coupling to phenylisothiocyanate (PITC) in mild base followed by cyclization in anhydrous acid.
   - ~~D.~~ Coupling to fluorodinitrobenzene (FDNB) in mild base followed by hydrolysis in 6 M HCl.

5. Why is a hydroxyl group a good hydrogen bond donor?
   - [✓ A.] The electronegativity of oxygen atoms is greater than hydrogen atoms.
   - ~~B.~~ The electronegative force between the oxygen and hydrogen is greater than that in a carbon-hydrogen bond.
   - ~~C.~~ The oxygen's unbonded pairs of electrons draw the electrons in the oxygen-hydrogen bond closer to the nucleus.
   - ~~D.~~ The partial charge on the oxygen atom makes it a good nucleophile.

6. 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.

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7. Which of the following statements about protein β (beta) structures is ***false***?
   - ✓A. The polypeptide chains are almost fully extended.
   - ~~B.~~ The hydrogen bonds are roughly perpendicular to the peptide backbone.
   - ~~C.~~ The hydrogen bonds link adjacent strands.
   - [✓ D.] The side-chain R-groups point away from the backbone, on one side of the beta sheet only.

8. 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.

9. The following sequence represents residues 163-171 of the human P450 1A2 protein. What secondary structure would be predicted for this sequence?

   WYF
   V I T
   C
   GPNDS

   GLU HIS VAL SER LYS GLU ALA LYS ALA
   α α β ↑ α α α α α

   - [✓ A.] alpha helix
   - ~~B.~~ beta strand
   - ~~C.~~ random coil (*i.e.*, no regular secondary structure)
   - D. the information given is not sufficient for a reliable prediction to be made

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

    Lys - Pro - Phe ↓- Val - Met - Lys - Trp ↓- His - Tyr ↓- Ala - Tyr - Pro - Cys - Arg - Leu

    1 2 3 4 5 6 7 8 9 10 11 12 13 14

    - [✓ A.] 3, 7 and 9
    - B. 6 and 14
    - C. 3, 7, 9 and 11
    - D. 1, 6 and 14

    Trp
    Phe
    Tyr

11. All of the following are considered "weak" interactions in proteins, *except:*
    - A. hydrogen bonds
    - B. disulfide bonds
    - C. hydrophobic interactions
    - ~~D.~~ van der Waals forces

12. 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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13. Why do the protein sequencing techniques developed by Sanger and Edman begin with raising the pH to about 9?
    - ~~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.

14. Which of the following interactions contributes most to the formation of secondary structure in proteins?
    - ~~A.~~ Hydrophobic effect
    - [✓ B.] Hydrogen bonding
    - ~~C.~~ van der Waals interactions
    - ~~D.~~ Electrostatic interactions between ion pairs

15. Linus Pauling's studies of the peptide bond showed that:
    - ~~A.~~ at pH 7, many different peptide bond conformations are equally probable.
    - ~~B.~~ peptide bonds are essentially planar, with no rotation about the C—N axis.
    - C. peptide bond structure is extraordinarily complex.
    - ~~D.~~ primary structure of all proteins is similar, although the secondary and tertiary structure may differ greatly.

16. The major reason that antiparallel β-stranded protein structures are more stable than parallel β-stranded structures is that the latter:
    - ~~A.~~ do not have as many disulfide crosslinks between adjacent strands.
    - B. do not stack in sheets as well as antiparallel strands.
    - C. have fewer hydrogen bonds between adjacent strands than antiparallel sheets.
    - [✓ D.] have weaker hydrogen bonds between adjacent strands.

17. The Nobel prize-winning experiments of C. Anfinsen on the denaturation and renaturation of the enzyme ribonuclease demonstrated that:
    - ~~A.~~ RNA, as well as protein, can have catalytic activity.
    - ~~B.~~ Oxygen is required for proteins to fold into their native conformations.
    - [✓ C.] The primary structure contains all the information required for secondary and tertiary structure of proteins.
    - ~~D.~~ Proteins fold into their native conformations in the cell, but cannot do so in the test tube.

18. Given that the plasma membrane of a red blood cell is about 7.5 nm (75 Å) thick, about how many turns of an alpha-helix in a membrane protein is needed to span the membrane?
    - ~~A.~~ 11
    - ~~B.~~ 14
    - [✓ C.] 21
    - ~~D.~~ 50

19

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19. The following peptide was analyzed as part of tandem mass spectrometry-based sequencing after trypsin digestion of a protein at pH 2.0, following the technique discussed in class:

Pro-Tyr-Asn-Ala-Pro-Ser-Thr-Arg

Using the list of masses of amino acids given below, what is the **mass** of the **third smallest** fragment recorded on the mass spectrometer?

Amino acid masses (Da)

~~G: 57.02147~~
S: 87.03203
T: 101.04768
P: 97.05276

R: 156.10111
~~H: 137.05891~~
Y: 163.06333
A: 71.03711

K: 128.09497
D: 115.02695
N: 114.04293

V: 99.06842
E: 129.04259
F: 147.06842

A. 257.14879 Da
B. 260.11609 Da
C. 344.18082 Da
D. 374.15902 Da

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

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

Using this reaction, the rate of breakdown of the enzyme-substrate complex can be described by the expression:

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

~~A.~~ k_1 ([E$_t$]-[ES])[S]
~~B.~~ k_2 [ES]
[✓ C.] k_{-1} [ES] + k_2 [ES]
~~D.~~ k_{-1} [ES]

21. In competitive inhibition, an inhibitor:
~~A.~~ binds at several different sites on an enzyme.
~~B.~~ binds covalently to the enzyme.
~~C.~~ binds *only* to the ES complex.
[✓ D.] binds reversibly at the active site.

22. What initial reaction velocity V$_0$ is observed if substrate concentration in an enzyme reaction is 0.5 x K$_M$ and V$_{max}$ is 2.4 x 10^{-6} mol L^{-1} min^{-1}?
A. 1.2 x 10^{-6} mol L^{-1} min^{-1}
B. 6.0 x 10^{-7} mol L^{-1} min^{-1}
~~C.~~ 2.4 x 10^{-6} mol L^{-1} min^{-1}
[✓ D.] 8.0 x 10^{-7} mol L^{-1} min^{-1}

$$V_o = \frac{V_{max}\,[S]}{K_m + [S]}$$

$$V_o = \frac{(2.4 \times 10^{-6})(0.5\ K_m)}{K_m + 0.5\,K_m}$$

$$= \frac{2.4 \times 10^{-6}\,(0.5\,\cancel{K_m})}{1.5\,\cancel{K_m}}$$

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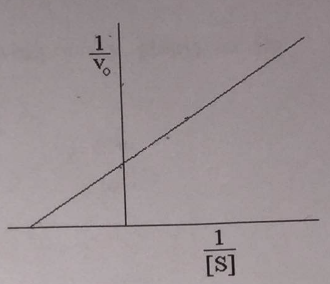
23. The figure shows a linear plot of kinetic data (a Lineweaver-Burke plot) for an enzyme catalyzed reaction. If the x-intercept is -3.3×10^3 L mol^{-1}, the y-intercept is 6.25×10^4 $Lmol^{-1}min$ and the slope is 18.75 min, what is the K_M of this reaction?

A. 1.6×10^{-5} $molL^{-1}$
[✓ B.] 3.03×10^{-4} $molL^{-1}$
C. 3.3×10^{3} $molL^{-1}$
D. 6.25×10^{4} $molL^{-1}$

$X\text{-int} = \frac{1}{-km}$



24. What concentration of a competitive inhibitor (K_i = 35 μM) is needed to produce an apparent K_M' that is 1.5 times the uninhibited K_M?

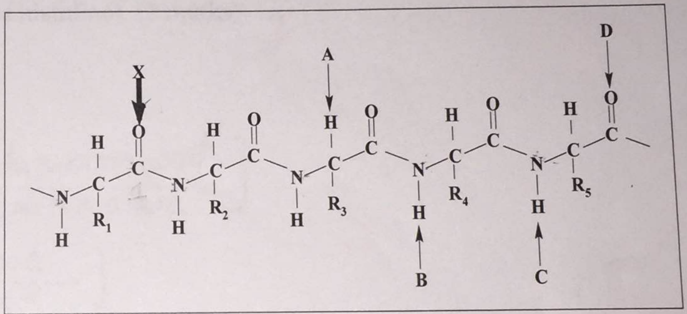
A. 17.5 μM
B. 52.5 μM
C. 35.0 μM
D. 57.5 μM

35 μm $\left(\frac{1 \times 10^{-6}}{1 \mu m}\right)$

35

25. A short section of alpha-helical peptide is shown in the figure at right. The O atom indicated by the heavy arrow (X) is hydrogen-bonded to the atom indicated by arrow ...

A. A
B. B
[✓ C.] C
D. D



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

Use the rest of this page for rough work

$Km = 3.03 \times 10^{-4}$ $k'm = 4.5 \times 10^{-4}$

$4.5 \times 10^{-4} = (3.03 \times 10^{-4})(1 + [I / 3.5 \times 10^{-5}])$

$1.485 = 1 + \frac{1}{3.5 \times 10^{-5}}$

$0.485 = \underline{[I]}$

M | 1

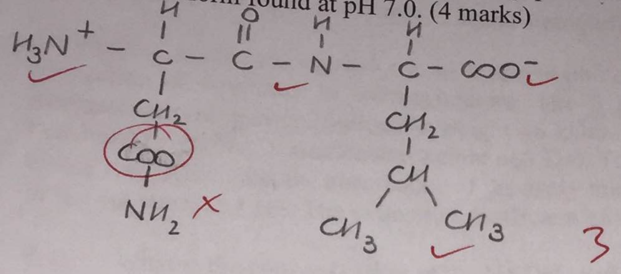
$[I] =$

Name ______________________________

Short answer questions (total marks = 25) **Student number** ____________________

1. Amino acids

Draw the complete structure of the dipeptide Asn-Leu (showing every atom) in its predominant form found at pH 7.0. (4 marks)



2. Ionization of amino acids

3-methylhistidine is a post-translationally modified form of histidine with a side chain **pK$_a$ of 5.8.** Calculate the net charge on 3-methylhistidine side chain at **pH 6.3** (The ionization states of 3-methylhistidine are same as that of histidine). (3 marks)

3

$pH = 6.3$

$pK_a = 5.8$

$$pH = pK_a + \log\left[\frac{\text{deprotanated}}{\text{protanated}}\right]$$

$$6.3 = 5.8 + \log\left[\frac{his}{his^+}\right]$$

$$\left[\frac{his}{his^+}\right] = 3.16$$

$$3.16 = \frac{\alpha}{1-\alpha} \begin{array}{l} \rightarrow \text{deprotanated} \\ \rightarrow \text{protanated} \end{array}$$

$$3.16 - 3.16\alpha = \alpha$$

$$\alpha = 0.76$$

$$1 - \alpha = 0.24$$

$$\therefore (0.76 \times 0) + (0.24 \times (+1)) = 0.24$$

The net charge on 3-methylhistidine side chain is + 0.24.

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3. Enzyme assay and activity

$$\text{isocitrate} + \text{NAD}^+ \xrightleftharpoons{\textit{isocitrate dehydrogenase}} \text{alpha-ketoglutarate} + \text{NADH} + \text{H}^+$$

An enzyme assay was carried out in a spectrophotometer cuvette at 340 nm to study the conversion of isocitrate to α-ketoglutarate. The 5 mL reaction mixture contained 20 µg isocitrate dehydrogenase (molecular weight 48 kDa), 2 mM isocitrate (molecular weight 192 Da) and 12 mM NAD$^+$ (molecular weight 663 Da). The total assay period was 15 min. At the end of the 15 minutes, the absorbance of the assay mixture increased by 0.32. The path length of the cuvette was 1 cm. The extinction coefficient of NADH is 6230 Lmol^{-1}cm^{-1}.

3.1) What is the **concentration** of the NADH product at the end of the assay period? *(Don't forget to include both the numerical value and the units, in your answer)* (2 marks)

$A = \ell \cdot c \cdot \varepsilon$

$0.32 = (1\,\cancel{\text{cm}})(c)\left(6230\,\frac{\text{L}}{\text{mol}\cdot\cancel{\text{cm}}}\right)$

$0.32 = \left(6230\,\frac{\text{L}}{\text{mol}}\right)(c)$

$c = \boxed{5.14 \times 10^{-5}\ \text{mol/L}}$

2

3.2) Calculate the **specific activity** of the above reaction in µmoles min^{-1} µg^{-1}. Show all your work for full marks. (3 marks)

Rate of reaction $= \frac{5.14 \times 10^{-5}\ \text{mol/L}}{15\ \text{min}}$

$= 3.43 \times 10^{-6}\ \text{mol/L}\cdot\text{min}$

3

Enzyme activity $= (3.43 \times 10^{-6}\ \text{mol/L}\cdot\text{min})(0.005\,\text{L})$

$= 1.7 \times 10^{-8}\ \text{mol/min}\left(\frac{1\ \mu\text{mol}}{1 \times 10^{-6}\ \text{mol}}\right)$

$= 1.71 \times 10^{-2}\ \mu\text{mol/min}$

Specific activity $= \frac{(1.71 \times 10^{-2}\ \mu\text{mol/min})}{20\ \mu\text{g}}$

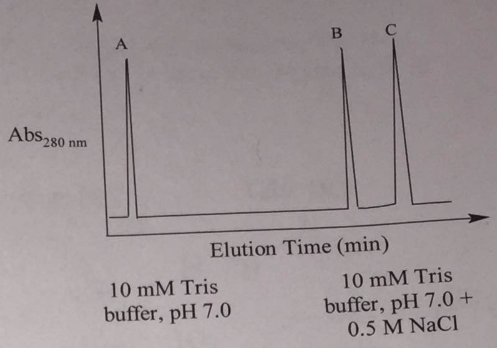
$\boxed{= 8.57 \times 10^{-4}\ \mu\text{mol/min}\cdot\mu\text{g}}$

Name ____________________ Student number ____________________

4. Chromatography

Binds to -ve

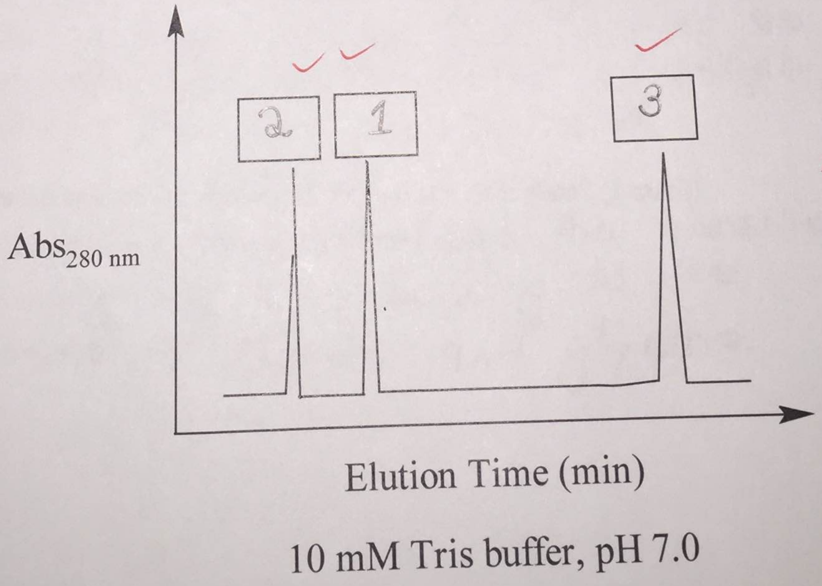
4.1) A mixture of peptides is separated using **anion** exchange chromatography (right). The peptides were eluted with the solutions indicated below the chromatogram and were detected by measuring the absorbance at 280nm. Identify which of the three peptides shown below corresponds to peaks A, B and C. (1.5 marks)



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

1.5

4.2) The chromatogram shown below was obtained when this same mixture was run through a gel filtration column. Assuming that the average weight of an amino acid residue is 110 Da, identify the peptide represented by each peak by indicating the number corresponding to the peptide in the box above the peak. (1.5 marks)



1.5

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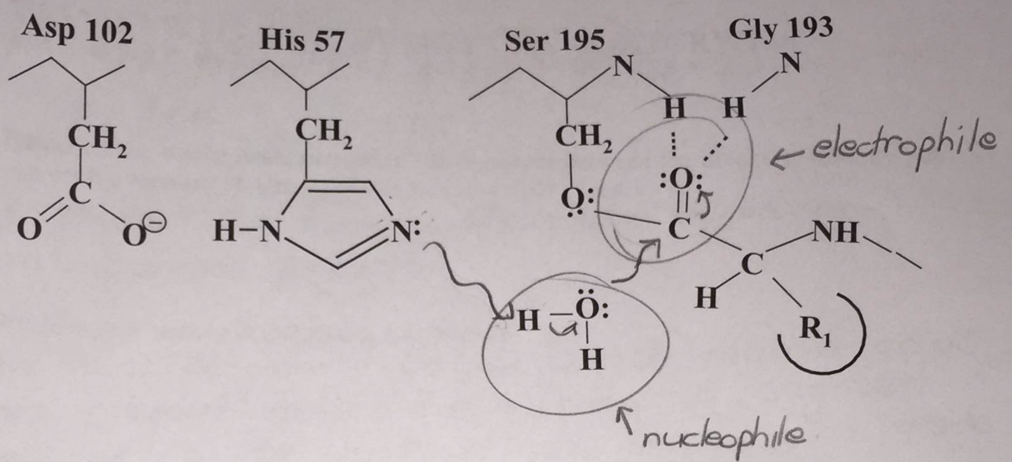
5. Chymotrypsin mechanism

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.



5.1) Label the atoms that act as the nucleophile and the electrophile in this step. (1 mark)

more specific 0.5

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

2

5.3) 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)

His-57 is acting as a general base and removing the Hydrogen from water so water can act as a better nycleophille to attack the C=O peptide bond. ✓ ✓ 1

5.4) What is the role of the oxyanion hole in this mechanism? (1 mark)

The oxyanion hole stabilizes the transition state by hydrogen bonding to the backbone of serine and glycine. 1

4

Name ______________________________ **Student number** ____________________

6. Protein tertiary structure

6.1) Draw a box around each cluster of secondary structure breakers in the peptide shown below. (1 mark) GPNDS   β WYF VITC

VEVQYTWTISAGNVHVTIQYTWNSKDITCRWTVQ
βαβαββββ ββ↑α βαβββαβββ α↑βββ α βββα

β – β – β

−1

6.2) Based on the amino acids present in the segments between the breakers, what do you predict the **tertiary structure** of this protein to be? (1 mark)

I predict the terhary structure to be an
anti-parallel β-barrel.

6.3) Explain your answer in question 6.2 (3 marks)

The local majority as to which amino acids
are present determine whether a segment
will be α or β. Most of the amino acids
found above prefer a β-structure because
of their large and bulkly side chains as
well as a side chain on the β carbon.
The peptide shown above has alternating
polar and non-polar amino acids and
thus forms this anti-parallel beta barrel.

−1