Name_____ Student number_____

UNIVERSITY OF GUELPH BIOC*4540 ENZYMOLOGY
Winter 2019 Midterm Examination: Feb 26, 2019 at 10:00 – 11:20 am in CRPS 116
Instructor: Prof R. Merrill

Instructions: **Time allowed = 80 minutes. Total marks = 40. The exam is 9 pages and consists of 29 questions. This midterm represents 20% of the final grade. Please write all your answers in ink, if possible. No materials may be removed from the examination room.**

Answers to Part A are to marked on the computer scoring sheet (see below) and answers to Part B are to be written directly on this examination paper. Answers to part C are to be written in the answer booklet provided.

Part A. On the computer scoring sheet provided, use **black lead pencil** to enter your name and your seven-digit student ID number.
Answer Part A, questions 1-23, on the computer-scoring sheet. Only one option is correct for each of these questions. Use **black lead pencil**. Erase cleanly if you make a mistake. **Do not use ink or white-out** on the computer-scoring sheet. *Twenty three (23) questions x 1 mark per question = 23 marks total.* **No marks will be deducted for incorrect answers.**

1. Kinesin pauses when it binds AMP-PNP because:

A) the nucleotide blocks entrance to the active site
B) the nucleotide covalently modifies the enzyme
C) the nucleotide binds to the active site but resists hydrolysis
D) the nucleotide binds to another site other than the active site and inhibits the enzyme
E) the nucleotide stabilizes an unfolded conformation of kinesin

2. Which statement of the following is correct for the nitrogenase enzyme complex:

A) it requires 10 electrons to fix each molecule of N_2
B) it possesses a pair of 6Fe-6S centers
C) it has a FeMn center with 7 Fe and 9 inorganic S atoms
D) it uses homocitrate to provide 2 oxygen ligands
E) it consists of 3 identical dinitrogenase reductase molecules

3. Phenylketonuria is caused by a genetic defect in:

A) phenylalanine hydroxylase
B) amino acid metabolism
C) tyrosine hydroxylase
D) aminotransferase
E) tetrahydrobiopterin synthesis

Name_____ Student number_____

4. The best description of group-specific enzymes is that they:

A) show non-specific activity against a variety of substrates
B) utilize one substrate only for a single reaction
C) use a variety of substrates each containing a certain functional group that is modified
D) exhibit high catalytic power
E) catalyze isomerization reactions

5. For enzymes in which the slowest (rate-limiting) step is the reaction

$$ES \xrightarrow{k_2} P$$

K_M becomes equivalent to:

A) k_{cat}
B) the [S], where $V_o = V_{max}$
C) the dissociation constant, K_d, for the ES complex
D) the maximum velocity
E) the turnover number

6. Which is NOT a reason behind the observation that a typical enzyme reaction decreases as incubation time increases:

A) denaturation of the enzyme
B) increase in the reverse reaction
C) inactivation of the catalytic residue(s)
D) product inhibition
E) inactivation of the coenzyme

7. The best definition of *catalytic center activity* for an enzyme is:

A) number of moles of substrate transformed into product per unit time per mole of enzyme under optimal conditions
B) number of moles of substrate transformed into product per unit time per mole of enzyme active sites under optimal conditions
C) an unimolecular kinetic activity for single substrate enzymes
D) number of moles of product transformed into substrate per unit time per mole of enzyme under optimal conditions
E) equals to V_{max} times $[E]_T$ per mole of enzyme active sites

8. Which of the following expressions is correct in the derivation of the Michaelis-Menten kinetic model?

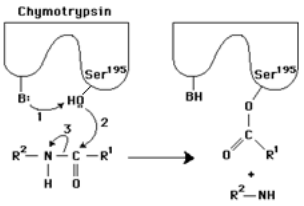
A) $k_2[S] - v_o [S] = K_M[E_T]$
B) $k_2[E_T] - v_o [S] = K_M[S]$
C) $k_2[E_T][S] - v_o [S] = K_M[S]$
D) $v_o[E_T][S] - k_2 [S] = K_M v_o$
E) $k_2[E_T][S] - v_o[S] = K_M v_o$

Name_____ Student number_____

9. Penicillin and related drugs inhibit the enzyme _____; this enzyme is produced by _____?

A) β-lactamase; bacteria
B) transpeptidase; human cells
C) transpeptidase; bacteria
D) lysozyme; human cells
E) aldolase; bacteria

10. In the following diagram of the first step in the reaction catalyzed by the protease chymotrypsin, the process of general base catalysis is illustrated by the number _____, and the process of covalent catalysis is illustrated by the number _____.



A) 2; 3
B) 1; 3
C) 1; 2
D) 2; 1
E) 3; 2

11. Which of these statements about enzyme-catalyzed reactions is *false*?

A) At saturating levels of substrate, the rate of an enzyme-catalyzed reaction is proportional to the enzyme concentration.
B) If enough substrate is added, the normal V_{max} of a reaction can be attained even in the presence of a competitive inhibitor.
C) The rate of a reaction decreases steadily with time as substrate is depleted.
D) The activation energy for the catalyzed reaction is the same as for the uncatalyzed reaction, but the equilibrium constant is more favorable in the enzyme-catalyzed reaction.
E) The Michaelis-Menten constant K_m equals the [S] at which $V = 1/2\ V_{max}$.

12. To determine K_m from the Hanes-Woolf transformation of the Michaelis-Menten equation you would:

A) take the x-axis intercept where $V_0 = 1/2\ V_{max}$.
B) take the reciprocal of the x-axis intercept.
C) multiply the x-axis intercept by –1.
D) take the reciprocal of the y-axis intercept.
E) multiply the y-axis intercept by –1.

Name_____ Student number_____

13. An uncompetitive inhibitor is

A) a substance that binds either to the free enzyme or to the E-S complex
B) a substance that binds to the E-S complex only
C) a substance that binds to the free enzyme only
D) a substance that only causes a change in the V_{max} for the reaction
E) none of the above

14. A drug must possess several features in order to show reasonable efficacy in the body. Which one of the following is NOT a pre-requisite for a drug candidate:

A) Must be chemically stable in the acidic environment of the stomach
B) Must be absorbed in the bloodstream and pass several membranes
C) Must bind tightly to substances in the body
D) Must survive a battery of enzymes in the liver
E) If the target is in the brain, it must pass the blood-brain barrier

15. In the covalent catalysis mechanism of amino transferases:

A) Pyridoxamine phosphate is the amino group acceptor
B) NH_2 from the amino acid substrate attacks the aldehyde of pyridoxal phosphate
C) The carbonyl of the α-keto acid attacks the Schiff base Lys-residue intermediate
D) OH^- attacks the amino acid C_α in the ketimine complex
E) none of the above.

16. The purpose of the bleaching laser beam in single molecule studies of β-galactosidase is:

A) To keep β-galactosidase in its oxidized state
B) To keep its substrate, resorcinol in the oxidized state
C) To excite the fluorescent substrate of β-galactosidase
D) To prevent the counting of the product molecules more than once each
E) To track the movement of the resorcinol substrate in the laser beam

17. A nonsequential enzyme mechanism is one in which

A) some substrates must become bound to the enzyme and some products must be released before other substrates become bound and other products are released
B) all of the substrates must become bound to the enzyme before any product is released
C) the reaction may be ordered or random
D) the conditions should be such that the reaction is zero-order with respect to the substrate
E) the reactants must undergo an unimolecular reaction with the release of a substrate or product

Name_____ Student number_____

18. Which is the correct equation for binding analysis involving fluorescence data

A) $\Delta F_{max} = \Delta F - K_d(\Delta F/[L])$
B) $F/F_0 = [L]/(K_d + [L])$
C) $\Delta F_{max} = \Delta F - K_d/([L]/\Delta F)$
D) $\Delta F = \Delta F_{max} - K_d([L]/\Delta F)$
E) $\Delta F = \Delta F_{max} - K_d(\Delta F/[L])$

19. Which of the following is a product of the cocaine esterase enzyme:

A) naphthalene
B) benzoic acid
C) egonine ester
D) phenol
E) coca cola

20. A metalloenzyme is an enzyme that

A) glows in the dark
B) binds a metal ion with micromolar affinity for structural purposes
C) catalyzes a redox reaction involving $FADH_2$
D) coordinates a metal ion with nanomolar affinity to provide reaction chemistry
E) uses a metal ion to stabilize an active site residue within the protein structure

21. Enzymes that cleave carbon-carbon bonds without the use of water in the chemical reaction are members of which class of enzymes?

A) oxidoreductases
B) transferases
C) hydrolases
D) lyases
E) isomerases

22. An enzyme can catalyze a reaction with either of two substrates, S_1 or S_2. The K_M for S_1 is 2.0 mM, and the K_M for S_2 is 20 mM. An Enzymology student determined that the V_{max} was the same for the two substrates. Unfortunately, he lost the page of his notebook and needed to know the value of V_{max}. He carried out two reactions: one with 0.1 mM S_1, the other with 0.1 mM S_2. However, he forgot to label which reaction tube contained which substrate. He obtained the following results:

Tube number	Rate of formation of product
1	0.5 μmol/min
2	4.8 μmol/min

The V_{max} for the enzyme is:

A) 91 μmol/min
B) 82 μmol/min
C) 20 μmol/min
D) 101 μmol/min
E) 200 μmol/min

Name_____ Student number_____

23. Which of the following is true for an assay to measure the concentration of enzyme in a sample:

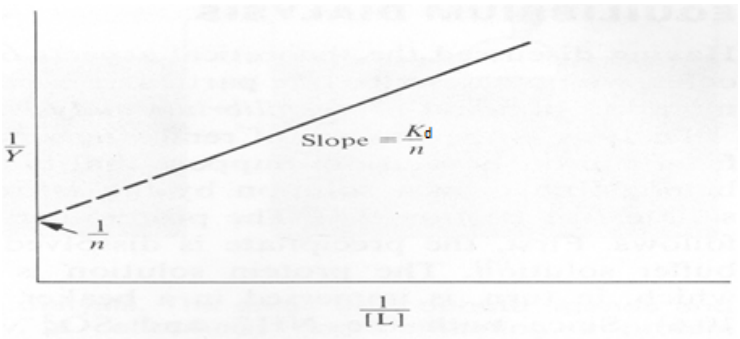
A) both [E] and [S] are present in limiting amounts
B) both [E] and [S] are present in excess amounts
C) the enzyme is present in much higher amount that the substrate
D) the conditions should be that the reaction is zero-order with respect to the substrate
E) none of the above

Name_____ Student number_____

B. **Part B. "Short answer" questions. Answer the following 4 questions (#24 – #27) with a short answer consisting of a few sentences (your answer can be in point form). Each question is worth 2 marks (8 marks for this section).**

24. Write out the equation for the Hughes-Klotz plot used for binding analysis and then sketch it. Be certain to label the axes and indicate the parameters that can be obtained from the slope and y-intercept (2 marks)

- $1/Y = 1/n + K_d/n[L]$

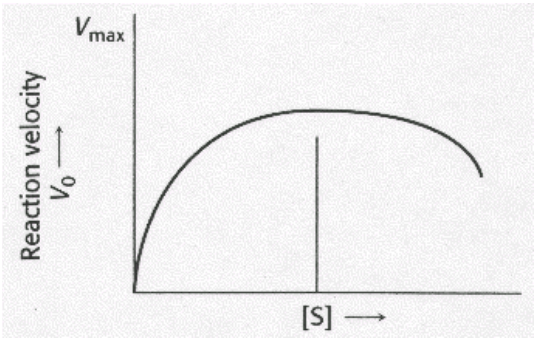


25. Define and explain the importance of "electrostatic interactions" in enzyme catalysis (2 marks).

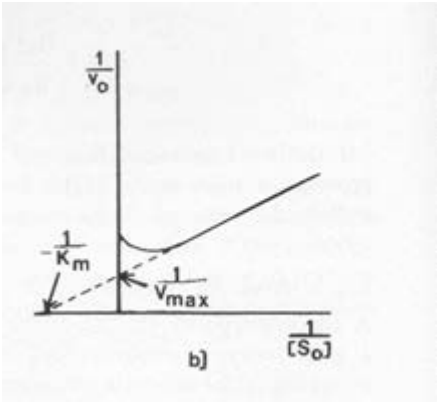
- Electrostatic interactions involve the stabilization of the distribution of electrical charge in transition states by strategically positioned charged residues within the active site of an enzyme. Water is also squeezed from the active site by the dynamics of the enzyme which allows it to intensify electrostatic interactions, including dipole-dipole associations. These types of interactions are very important for stabilizing the transition state for a reaction and hence lead to large increases in the catalytic rate of the reaction. An example would be to locate a Lys (+) residue near the oxygen atom of a carbonyl group or a Glu (-) near the carbonyl carbon to favour the formation of the tetrahedral intermediate for the reaction.

Name_____ Student number_____

26. A simple Michaelis-Menten enzyme, in the absence of any inhibitor, displayed the following kinetic behaviour. The expected value of V_{max} is shown on the y-axis. (a) Write out a reaction scheme that would describe this type of kinetic behavior (1 mark). (b) Sketch a Lineweaver-Burk plot that corresponds to the velocity-versus-substrate curve (1 mark).



$$E + S \Leftrightarrow ES \rightarrow E + P$$
$$+S \downarrow\uparrow -S$$
$$SES$$



27. What are transition state analogues and why do they generally show competitive inhibition for a given enzyme-catalyzed reaction? Why do they also exhibit high binding affinity to the enzyme? (2 marks)

- Transition-state analogues are molecules that are similar to the transition state species for a given chemical reaction except that the analogue will **not function** as a substrate for the target enzyme.

- Transition-state analogues are mimics of the transition state species and so they should be able to out-compete the substrate for the reaction (competitive inhibitors).

- These analogues should also bind the enzyme **more tightly** than does the substrate in the E-S complex because these **fit the active site better** than the substrate, with more noncovalent interactions including H-bonds, van der Waals contacts, electrostatic and hydrophobic interactions, etc (higher binding affinity).

Name_____ Student number_____

Part C. "Problem-based" questions. Answer the following 2 questions in the <u>examination booklet</u> provided. Please write your answers in pen (not red ink). Question #28 is worth 5 marks and #29 is worth 4 marks. The two questions are worth a total of 9 marks.

28. You are studying the bacterial enzyme, catalase, MW = 247,500 g/mol. You manage to extract 10 mg of enzyme material from a 1L of bacterial cell culture. However, the enzyme solution is not pure, as you find (from SDS-PAGE and nucleic acid analysis) that it contains some other proteins as well as DNA. You perform a nucleic acid test and determine that 10% of the material is DNA (the rest is proteinaceous matter). You remove 5 mg from the original vial and transfer it to a new vial. You add 2.0 mL of buffer to this vial. You then take 200 µL of this solution and make a solution totalling 10 mL. Now, you remove 50 µL from this solution and then you add 1950 µL of buffer. From this solution you remove three, 10 µL aliquots and assay for O_2 production using an O_2-selective probe. You find that these samples produce 7.1, 6.8, and 6.4 µg of O_2 (MW = 32 g/mol) in the 5-minute assay. (a) What is the specific activity of the original solution (the first solution that you made)? (b) How many units of activity are present in the 10 mg of original extracted material? (c) How many katals are present in the original material?

- 10 mg of material (90% is protein)
- remove 5 mg put in 2 mL ∴ 2.5 mg/mL x 0.90 = 2.25 mg/mL protein = **stock 1**
- remove 200 µL and make to 10000 µL (50-fold dilution) = 0.045 mg/mL **stock 2**

remove 50 µL and make to 2000 µL (40-fold dilution) = 1.125 x 10^{-3} mg/mL =**stock 3**

- remove 10 µL for assay
  - 3 samples (7.1 + 6.8 + 6.4 µg /3 = 6.77 µg O_2 product; average)
  - 6.77 µg x 1 µmol/32 µg = 0.212 µmol product in 10 µL of stock 3
  - 0.212 µmol O_2 /5 min = 0.0424 µmol/min = 0.0424 U
  - How much protein in assay sample: 1.125 x 10^{-3}mg/mL x 0.010 mL = 1.125 x 10^{-5}mg
  - The **specific activity of the original stock 1** is the same since the stocks are only diluted with buffer = 0.0424U/1.125 x 10^{-5} mg = 3769 U/mg protein

(b) How many Units are in the 10 mg of original extracted material?

Units/mg protein = 3769 U/mg protein x 9 mg protein in original powder = 33921 U

c) How many katals are present in the original material?

- **Katal** = 1 mol/s; 33921 µmol/min x 1 min/60s x 1mol/10^6 µmol = 5.65 x 10^{-4} katals

Name_____ Student number_____

29. Glutamate is a competitive inhibitor of pyruvate carboxylase and it reduces the enzyme activity by 75%. (a) If the pyruvate (substrate) concentration for the enzyme was 3.5 x 10^{-5} M and the K_M for this substrate is 4.4 x 10^{-6} M, what was the initial glutamate concentration (K_i = 2.4 x 10^{-7} M)? (b) What concentration must pyruvate be raised to in order to restore the velocity of the enzyme to its uninhibited value?

(a) Work the problem in terms of v_o and V_{max}

$v_o = V_{max}[S]/K_M + [S] = V_{max}(3.5 \times 10^{-5}M)/(4.4 \times 10^{-6}M + 3.5 \times 10^{-5}M) = V_{max}(3.5 \times 10^{-5}M)/3.94 \times 10^{-5}M = 0.89V_{max}$

$\therefore v_o = 0.89V_{max}$ and substitute for v_o where at 75% inhibition: $v_i = 0.25\ v_o$ $\therefore v_i = 0.25(0.89\ V_{max}) = 0.2225\ V_{max}$;

so $v_i/V_{max} = 0.2225$

$$v_i/V_{max} = \frac{[S]}{K_M(1+\frac{[I]}{K_i}) + .[S]} = \frac{3.5.x.10^{-5}M}{[(4.4.x.10^{-6}M)(1+\frac{[I]}{2.4.x.10^{-7}M}) + 3.5.x.10^{-5}M]}$$

0.2225 =

$$\frac{3.5.x.10^{-5}M}{[(4.4.x.10^{-6}M) + (\frac{4.4.x.10^{-6}M * [I]}{2.4.x.10^{-7}M}) + 3.5.x.10^{-5}M]} = \frac{3.5.x.10^{-5}M}{18.33[I] + 3.94.x.10^{-5}M}$$

$4.078[I] + 8.77 \times 10^{-6}M = 3.5 \times 10^{-5}M$

$4.078[I] = 2.62 \times 10^{-5}M$ $\therefore [I] = 6.42 \times 10^{-6}M$

(b) To overcome the inhibition then $v_i = v_o$ $\therefore v_i = 0.89\ V_{max}$ and $v_i/V_{max} = 0.89$

$$[S] = 0.89\ (4.4 \times 10^{-6}M\ (1+\frac{6.42.x.10^{-6}M}{2.4.x.10^{-7}M}) + [S])$$

$[S] = 3.92 \times 10^{-6}M + 1.050 \times 10^{-4}M + 0.89[S]$

$0.11[S] = 1.089 \times 10^{-4}M$

$[S] = 9.90 \times 10^{-4}M$