Name_____ Student number:_**ANSWER KEY**_____

UNIVERSITY OF GUELPH BIOC*4540 ENZYMOLOGY

Winter 2020 Midterm Examination: February 25, 2020 at 10:00 – 11:20 am in CRPS 116

Instructor: Prof R. Merrill

Instructions: **Time allowed = 80 minutes. Total marks = 40. The exam is 8 pages and consists of 30 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 be 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. Which statement is correct about extreme homoallostery in enzymes:

   A) substrate can accumulate to $[S]_c$ but at higher concentrations it slows down
   B) at concentrations below $[S]_c$, the enzyme is almost inactive
   C) at concentrations above $[S]_c$, the enzyme is not very efficient
   D) the sigmoidal curve has a shallow slope until the enzyme reaches V_{max}
   E) none of the above

2. Which of the following is NOT true for the reaction mechanism of chymotrypsin:

   A) it is an example of covalent catalysis
   B) the rate-limiting step is the acylation of the enzyme
   C) the enzyme uses an active-site serine as the nucleophile for the reaction
   D) low barrier hydrogen bonds are important for the reaction
   E) the enzyme prefers to hydrolyze peptide bonds adjacent to an aromatic residue

3. The Scatchard equation for binding analysis of data for n equivalent sites is:

   A) $Y/[L] = n/K_d + Y/K_d$
   B) $n/[L] = Y/K_d – Y/[L]$
   C) $[L]/Y = n/K_d – Y/[L]$
   D) $Y/[L] = n/K_d – Y/K_d$
   E) $[L]/Y = Y/K_d – n/K_d$

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4. Which of the following strategies is NOT typical for enzyme catalyzed reactions:

A) enzymes position the substrate near catalytic residues
B) chemical bonding may occur between the enzyme and substrate
C) protons transfer between the enzyme and substrate atoms
D) electrons may be transferred between enzyme and substrate atoms
E) highly stable complexes between the enzyme and substrate are established

5. The role of Ni^{2+} in urease is to:

A) polarize the c=O in the urea substrate for nucleophilic attack
B) stabilize the protein to prevent denaturation
C) complex H_2O as substrate for the hydrolysis reaction
D) act as the nucleophile for the reaction
E) act as an electrophile for the reaction

6. In the nitrogenase enzyme:
(will accept either B or D answers here)

A) the P-cluster consists of a pair of 6Fe-6S centers that share a S atom
B) the FeMo center consists of 7 Fe and 9 inorganic S atoms
C) has a Mo atom with ligands to 4 inorganic S atoms
D) 10 electrons are required to fix each molecule of N_2
E) 2 nonidentical dinitrogenase heterodimers form the complex with 2 nonidentical dinitrogenase reductase molecules

7. In the derivation of the Michaelis-Menten equation, which statement is true:

A) $[E]_T[S] - [ES][S] = K_M[ES]$
B) $[E]_T[S] - [ES]/[S] = K_M[ES]$
C) $[E]_T[S] + [ES]/[S] = K_M[ES]$
D) $K_M[E]_T = [E]_T[ES] + [ES]$
E) none of the above

8. In denim finishing, cellulases are used to:

A) release some terpene dye from the surface to produce a bleached effect
B) photo-oxidize the blue denim material to lighten the color
C) break the amide bonds in the denim to give a faded appearance
D) release some indigo dye to give a random faded pattern
E) break ether links to release the blue dye from the denim to produce the desired effect

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9. An noncompetitive 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 causes a change in V_{max} and K_M for the reaction
E) none of the above

10. Which of the following statements is *false*?

A) A reaction may not occur at a detectable rate even though it has a favorable equilibrium.
B) After a reaction, the enzyme involved becomes available to catalyze the reaction again.
C) For S $\rightarrow$ P, a catalyst shifts the reaction equilibrium to the right.
D) Lowering the temperature of a reaction will lower the reaction rate.
E) Substrate binds to an enzyme's active site

11. For enzyme quantum tunneling to occur:

A) the temperature of the reaction must be near absolute zero
B) the substrate must tunnel into the enzyme active site
C) covalent catalysis must be the mechanism used by the enzyme
D) the geometry of the protein must be distorted
E) the enzyme must bind the substrate(s) with high affinity

12. The explanation for catalytic heterogeneity for a single enzyme molecule is:

A) large numbers of conformers with highly variable catalytic powers exist for a single enzyme molecule and interconvert rapidly
B) large numbers of conformers with highly variable catalytic powers exist for a single enzyme molecule and interconvert slowly
C) small numbers of conformers with highly variable catalytic powers exist for a single enzyme molecule and interconvert slowly
D) small numbers of conformers with highly variable catalytic powers exist for a single enzyme molecule and interconvert rapidly
E) some enzyme molecules are denatured in solution and so do not contribute to the activity

13. Which of the following is true of the binding energy derived from enzyme-substrate interactions?

A) It can always provide enough energy to explain the large rate accelerations brought about by enzymes.
B) It is sometimes used to hold two substrates in the optimal orientation for reaction.
C) It is the result of covalent bonds formed between enzyme and substrate.
D) Most of it is derived from covalent bonds between enzyme and substrate.
E) Most of it is used up simply by binding the substrate to the enzyme.

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14. The benefit of measuring the *initial rate* of a reaction v_0 is that at the beginning of a reaction:

A) $v_0 = V_{max}$
B) [ES] can be measured accurately
C) varying [S] has no effect on v_0
D) changes in [S] are negligible, so [S] can be treated as a constant
E) changes in K_m are negligible, so K_m can be treated as a constant

15. According to Absolute Rate Theory, the rate constant (k) of a chemical reaction increases:

A) linearly with temperature
B) inversely with temperature
C) exponentially with $\Delta\Delta G^{\ddagger}$
D) exponentially with $\Delta G^{\ddagger}$
E) inversely with substrate concentration

16. Which of the following is the correct expression for the Turnover Number for an enzyme?

A) TN = μmol(S→P) x min^{-1} x mL^{-1}/ (μmol enzyme or cat. site x mL^{-1})
B) TN = μmol(S→P) x min^{-1} x mL/ (μmol enzyme or cat. site x mL^{-1})
C) TN = μmol(S→P) x min x mL^{-1}/ (μmol enzyme or cat. site x mL^{-1})
D) TN = μmol(S→P) x min x mL/ (μmol enzyme or cat. site x mL^{-1})
E) TN = μmol(S→P) x min^{-1} x mL^{-1}/ (μmol enzyme or cat. site x mL)

17. The best substrate for an enzyme is one that:

A) has the highest V_{max} and the highest K_M values
B) has the lowest V_{max} and the highest K_M values
C) has the highest V_{max} and the lowest k_{cat} values
D) binds with the highest affinity to the enzyme active site
E) has the highest V_{max} and the lowest K_M values

18. A metal-activated enzyme is one that

A) binds transition metals irreversibly
B) binds a metal ion with micromolar affinity
C) catalyzes a redox reaction involving $FADH_2$
D) coordinates a metal ion with picomolar affinity to provide reaction chemistry
E) uses a metal ion to prevent oxidation of its substrate

19. The ED_{50} for a drug candidate is:

A) mean lethal dose required to kill 50% of a test sample
B) the concentration needed to reduce an enzyme activity to 50% of its maximal value
C) the effective dose to produce a particular toxic effect in one-half of the animals
D) the effective dose to produce a therapeutic effect in 50% of a test sample
E) the effective dose to produce a particular toxic effect in all of the animals

20. Which of the following statement(s) about enzyme reaction transition states is/are true:

A) they show lifetimes that occur over a wide range of time scales
B) they possess greater complementarity with the enzyme active site than do the substrates or products
C) can be captured by spectroscopic techniques
D) were conceptually conceived by William Jencks and experimentally verified by Linus Pauling
E) all of the above

21. For covalent catalysis involving aminotransferase enzymes the following is correct:

A) an active site lysine acts as the amine donor
B) alpha-ketoglutarate is one of the products for the reaction
C) the enzyme is present in much higher amount that the substrate
D) involves an amine attack on an imine carbon
E) all of the above

22. Which is NOT a feature of Biotouch™ Detergent enzymes now commonly used for laundry detergent:

A) may be a cysteine protease
B) shows good activity in soft water
C) effective at temperatures between 20 - 40°C
D) improved enzyme stability
E) may be a serine protease

23. Catalytic antibodies

A) usually are more efficient enzymes than the natural enzyme they are designed to mimic
B) are also known as covalent antibodies
C) are prepared by using an antigen that consists of a covalently attached transition-state analogue species to a carrier protein
D) were conceptually conceived by William Jencks and experimentally verified by Linus Pauling
E) can be used to activate enzymes

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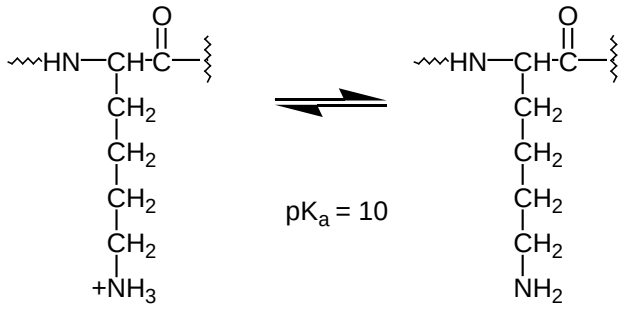
Part B. "Short answer" questions. Answer the following 5 questions (#24 – #28) with a short answer consisting of a few sentences (your answer can be in point form). You may write your answer directly in the space below each question. Each question is worth 2 marks (10 marks in total for this section).

24. Briefly explain the nature of the expected pK_a shift of the following active site residue in an enzyme. State the value for the unaltered pK_a and the direction (higher or lower) of the expected shift and why. You may draw diagrams and/or ionization schemes to help answer this question. A lysine residue in a nonpolar active site pocket. The normal pK_a for the lysine residue in proteins is approx. 10 and it is in equilibrium with its salt and acid forms.



A nonpolar pocket (environment) will favour the basic form of the Lys residue ($-NH_2$) and so this will shift the pK_a to lower pH values because it will be harder to protonate the side chain or, put another way, the Lys side chain will have a lower affinity for H^+ in this environment because the uncharged (deprotonated) species is favoured.

25. Briefly describe how gel filtration can be used to determine the binding constant between an enzyme and one of its substrates.

- use a size-exclusion resin such as Sephadex, Sephacryl, or Biogel and can separate the free ligand (substrate) from the enzyme-substrate complex
- equilibrate the column in the appropriate concentration of substrate and then inject the enzyme sample into the column and run it to determine the peak where the enzyme comes out and the [S] in that peak
- repeat for each [S] and then determine bound versus free [S] and plot to calculate the K_D

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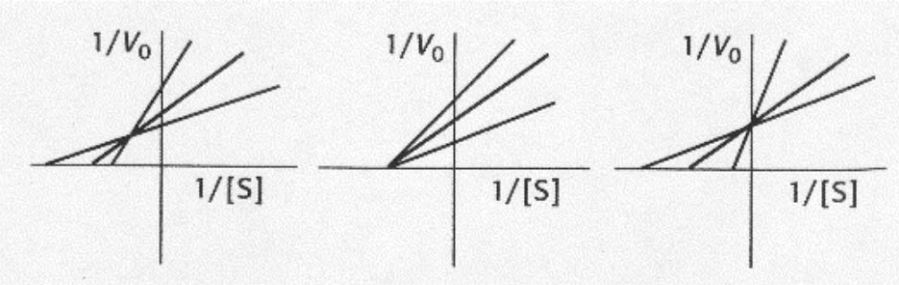
26. For a single-substrate enzyme-catalyzed reaction, double reciprocal plots were determined for three different enzyme concentrations. Which of the following three families of curves (curves 1, 2 or 3) would you expect to be obtained for an enzyme that obeys Michaelis-Menten kinetics? Explain.



1) 2) 3)

Plot #2 **would be expected** for an enzyme that obeys Michaelis-Menten kinetics for three different enzyme concentrations. If $[E]_T$ is increased, then V_{max} will also increase because $V_{max} = k_2[E]_T$. But, $K_M = (k_{-1} + k_2)/k_1$, i.e., K_M is independent of $[E]_T$. Plot 1 shows that both K_M and V_{max} change and plot 3 shows that V_{max} doesn't change while K_M does. Plot 2 is correct because it shows that K_M doesn't change while V_{max} does.

27. What are low-barrier H-bonds and how does an enzyme use them to lower the ΔG* for a reaction? (2 marks)

When the barrier to H-exchange between an H-bond donor and acceptor has reduced to the **zero point** energy level for hydrogen the interaction is referred to as a **low-barrier H-bond (LBHB).** This results in an equal sharing of the H atom between donor and acceptor and a special resonance H-bond forms. This new H-bond is more stable than the original (60 kJ/mol compared with 20 kJ/mol) and the energy released in forming the LBHB may be used to help the reaction which forms it by lowering the activation barrier for the reaction (ΔG*). This is therefore one mechanism whereby an enzyme can lower the ΔG* for a reaction.

28. What are coupled enzyme reactions and how would you set up such a system?

-coupled reactions--some reactions are difficult to assay because of the absence of a readily measurable compound or property
- couple one reaction to another which produces a measurable product
-example is the hexokinase reaction coupled with glucose-6-P-dehydrogenase which would allow the monitoring of the hexokinase rate by the appearance of NADPH produced by the coupled enzyme, G6P-dehydrogenase.

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Part C. "Problem-based" questions. Answer the following 2 questions in the examination booklet provided. Please write your answers in pen (not red ink). Question #29 is worth 4 marks and #30 is worth 3 marks. The two questions are worth a total of 7 marks.

29. Methotrexate is a competitive inhibitor of dihydrofolate reductase and it was found to cause 70% inhibition of the enzyme's activity. (a) If the tetrahydrofolate (substrate) concentration for the enzyme was 3.5×10^{-5} M and the K_M for this substrate is 4.4×10^{-6} M, what was the initial methotrexate concentration ($K_i = 2.4 \times 10^{-7}$ M)? (b) What concentration must tetrahydrofolate 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 70% inhibition: $v_i = 0.30\ v_o \ \therefore v_i = 0.30(0.89\ V_{max}) = 0.267\ V_{max}$

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

$$0.267 = \frac{3.5.x.10^{-5}M}{[(4.4.x.10^{-6}M) + (\frac{4.4.x.10^{-6}M * [I]}{2.4.x.10^{-7}M}) + 3.5.x.10^{-5}M]} = \frac{3.5.x.10^{-5}M}{18.33[I] + 3.94.x.10^{-5}M}$$

$4.90[I] + 1.05 \times 10^{-5}M = 3.5 \times 10^{-5}M$

$4.90[I] = 2.45 \times 10^{-5}M \qquad \therefore [I] = 5.0 \times 10^{-6}M$

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

$$v_i/V_{max} = \frac{[S]}{4.4.x.10^{-6}M(1+\frac{5.0.x.10^{-6}M}{2.4.x.10^{-7}M}) + [S]} = 0.89$$

$$[S] = 0.89\ (4.4 \times 10^{-6}M\ (1+\frac{5.0.x.10^{-6}M}{2.4.x.10^{-7}M}) + [S])$$

$[S] = 0.89\ (4.4 \times 10^{-6}M + 9.17 \times 10^{-5}M + [S])$

$[S] = 3.92 \times 10^{-6}M + 8.16 \times 10^{-5}M + 0.89[S]$

$0.11[S] = 8.55 \times 10^{-5}M$

$[S] = 7.78 \times 10^{-4}$ M

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30. Succinate dehydrogenase was assayed at an initial succinate concentration of 7.2×10^{-4} M. In 4 minutes, 30% of the succinate had been consumed.

The K_M for succinate is 2.5×10^{-2} M.

Succinate + FAD <--> Fumerate + $FADH_2$

Calculate:
(a) k (rate constant)
(b) V_{max}
(c) the concentration of fumerate produced by 9 minutes.

(a) $[S]_0 = 7.2 \times 10^{-4}$M << K_M therefore reaction is first order w.r.t. succinate (substrate) concentration.

For a first order reaction: $\ln([S_o]/[S_t]) = kt$; $\ln(100/70) = k$ (4 min); $k = 0.089\ min^{-1}$

(b) Recall from lecture notes: $k = V_{max}/K_M$ for a first order reaction (simplification of the Michaelis-Menten equation) so then $V_{max} = k(K_M)$

$V_{max} = (0.089\ min^{-1})(2.5 \times 10^{-2}M) = 2.23 \times 10^{-3}M\ min^{-1}$

(c) For a first order reaction (log $base_{10}$): $2.303 \log([S]_0/[S]_t) = kt$ (integrated Michaelis-Menten equation for a first order reaction).

$2.303 \log (7.2 \times 10^{-4}\ M/[S]_t) = 0.089\ min^{-1}(9\ min)$
$2.303 (\log 7.2 \times 10^{-4} - \log [S]_t) = 0.801$
$\log 7.2 \times 10^{-4} - \log [S]_t = 0.348$ M
$(0.857 - 4) - \log [S]_t = 0.348$M
$-\log [S]_t = 3.491$ M
$1/[S]_t = 3.10 \times 10^3$ M
$[S]_t = 3.23 \times 10^{-4}$ M

Thus, $[fumerate]_9 = [S]_0 - [S]_t = (7.2 \times 10^{-4}\ M) - (3.23 \times 10^{-4}\ M) = 3.97 \times 10^{-4}\ M$