A

Name: _____**Answer Key**_____
Last name, First name

Student Number: _____

BCH2333B Term Test #3

Il est interdit de se servir de téléphones cellulaires, de dispositifs électroniques non autorisés ou de notes de cours (à moins qu'il s'agisse d'un examen à livre ouvert). Les téléphones et les dispositifs doivent être fermés et rangés dans votre sac : vous ne pouvez pas les laisser dans vos poches ou sur votre personne. Sinon, on pourrait vous demander de quitter l'examen immédiatement et des allégations de fraude scolaire pourraient être déposées dont le résultat pourrait être un 0 (zéro) pour l'examen.

En apposant votre signature, vous reconnaissez vous être assuré de respecter l'énoncé ci-dessus.

Cellular phones, unauthorized electronic devices or course notes (unless an open-book exam) are not allowed during this exam. Phones and devices must be turned off and put away in your bag. Do not keep them in your possession, such as in your pockets. If caught with such a device or document, the following may occur: you will be asked to leave immediately the exam, academic fraud allegations will be filed which may result in you obtaining a 0 (zero) for the exam.

By signing below, you acknowledge that you have ensured that you are complying with the above statement.

Signature

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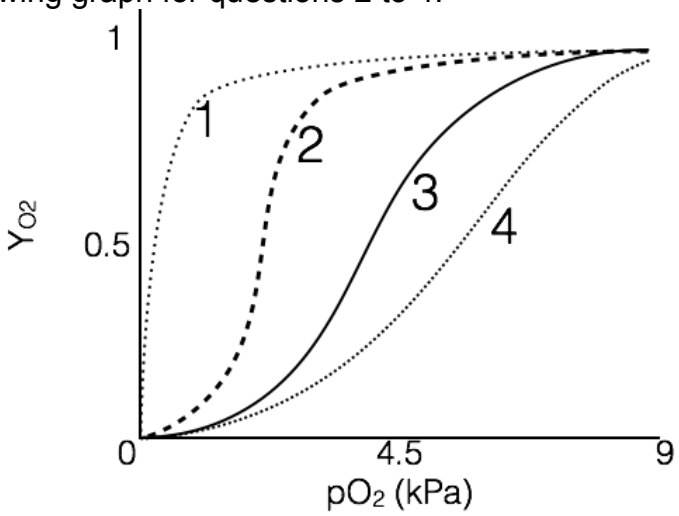
There is only **one** correct answer per multiplechoice question. Select the correct answer. **[1 mark each]**

1. The protein calcineurin binds to calmodulin with an association rate constant of $8.9x10^3$ M^{-1} s^{-1} and an overall dissociation constant (K_D) of 10 nM. The dissociation rate constant for this binding pair is:

a. $8.9x10^4$ M s^{-1}
b. $8.9x10^{-11}$ s^{-1}
c. $8.9x10^{-5}$ s^{-1}
d. $8.9x10^{11}$ M^{-2} s^{-1}
e. $8.9x10^5$ s^{-1}

Refer to the following graph for questions 2 to 4.



2. Adult hemoglobin has a P_{50}=4 kPa, which corresponds to curve:

a. 1
b. 2
c. 3
d. 4
e. Unable to determine

3. Fetal hemoglobin binds O_2 stronger than adult hemoglobin, but still exhibits allosteric binding of ligand, which is represented by curve:

a. 1
b. 2
c. 3
d. 4
e. Unable to determine

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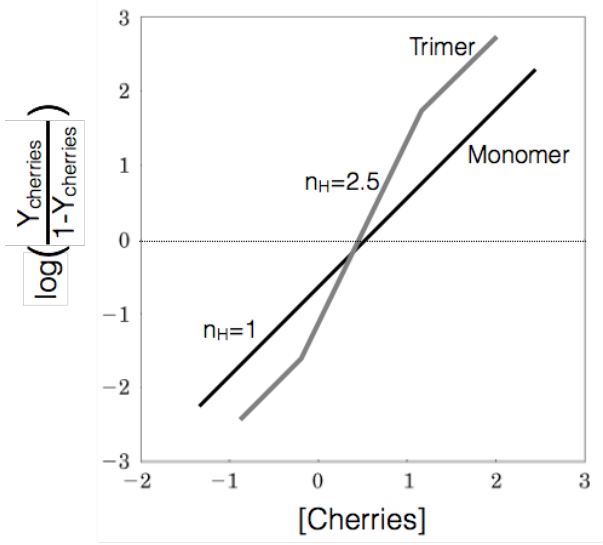
4. Considering that fetal oxygen is sourced from maternal blood, where the P_{O2} in blood is less than the P_{O2} in the mother's lungs, the properties of fetal hemoglobin:

a. Are an adaptive mechanism that allow maximal fetal hemoglobin loading from maternal blood.
b. Prevent the fetal hemoglobin from removing all of the oxygen from the mother's blood.
c. Promote acidosis in the placenta to induce the Bohr Effect.
d. Are a form of functional regulation of hemoglobin through isozymes.
e. Both a and d.

An enzyme tortase converts cherries into pie. Tortase can exist as a monomer or a homotrimer, where the trimer is favoured in people living in geographic regions with a tragic lack of pies. Note that each subunit has an active site. Questions 5-7 relate to the following Hill plot of monomeric (black line) and trimeric (grey line) tortase.



5. From the Hill plot, the trimerization of tortase results in:

a. negative cooperativity
b. random cooperativity
c. no cooperativity
d. positive cooperativity
e. sequential cooperativity

6. How many fold easier is the binding of the last cherry by tortase than the first cherry?

a. 32
b. 1.5
c. 2.5
d. 316
e. 3

7. The Hill coefficient of trimeric tortase is less than the total number of binding sites, indicating that:

a. Tortase is an inefficient enzyme.
b. Tortase must not exist as a trimer
c. Cherries do not bind simultaneously to all active sites of tortase
d. The Hill plot has a mistake in it
e. Tortase uses other berries for its pies

8. Which of the following regarding enzyme catalysis is incorrect?

a. The free energy of the transition state is larger than the free energy of the intermediates.
b. Enzymes change the overall ΔG of the reaction.
c. Enzymes work by lowering the energy barrier required to reach the transition state through transition state stabilization.
d. $\Delta\Delta G^{\circ\ddagger}$ is a measure of how much the transition state is being stabilized by the enzyme.
e. Once the transition state forms, it decays into product at a rate according to the bond vibrational constant A.

9. Which of the following would lead to a decrease in binding affinity?

a. Releasing more protein-bound water molecules upon binding
b. Decreasing the number of rotational degrees of freedom of the target ligand upon binding
c. Adding more hydrophobic interactions to the ligand-protein interface
d. Increasing the number of hydrogen bonds between the ligand and protein
e. Increasing the alignment of cationic atoms of the ligand with anionic atoms of the protein

10. In a single substrate enzyme-catalyzed reaction, the forward rate constant (formation of ES) is $4.3 \times 10^{6}\ M^{-1}s^{-1}$, the reverse rate constant (dissociation of ES to E +S) is $2.4 \times 10^{2}\ s^{-1}$, and the catalytic rate constant (turnover of ES to P) is $1.2 \times 10^{3}\ s^{-1}$. From this data, K_M is:

a. $1.79 \times 10^{4}\ M^{-1}s^{-1}$
b. $5.58 \times 10^{-5}\ M$
c. $1.79 \times 10^{4}\ M$
d. 5
e. $3.35 \times 10^{-4}\ M$

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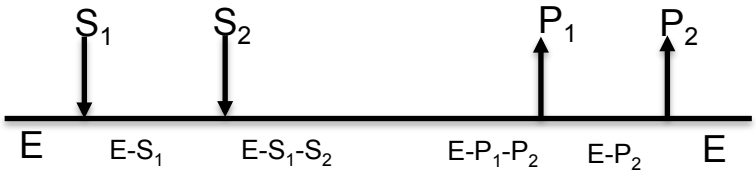
For questions 11 and 12, refer to the following Cleland Plot:



11. The bisubstrate enzyme-catalyzed reaction shown is:

a. Sequential Random
b. Ping-Pong
c. Allosteric
d. Sequential Ordered
e. Sequential Ternary

12. For this type of enzyme-catalyzed reaction, the following changes would be expected on a Lineweaver-Burke plot if [S_1] was held constant and [S_2] was varied:

a. The slope would decrease
b. The slope would increase
c. The x-intercept would increase
d. The y-intercept would increase
e. Both x and y-intercepts would increase but the slope would be unchanged

13. All reversible inhibitors that form the ternary enzyme-substrate-inhibitor (EIS) complex:

a. Will have a larger apparent K_M
b. Will have a smaller apparent K_M
c. Will have a larger apparent V_{max}
d. Will have a smaller apparent V_{max}
e. Will restore the apparent K_M to the uninhibited K_M at high substrate concentrations.

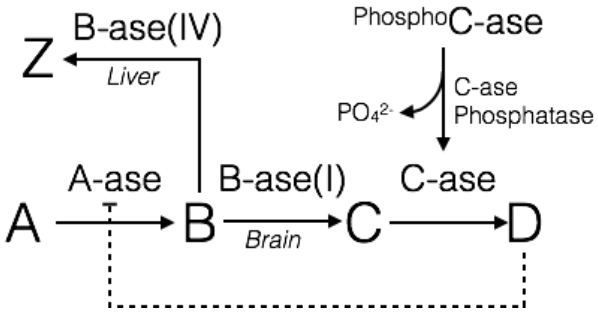
14. The correct order, from *least to most* specific, of irreversible inhibitors in terms of enzyme specificity is:

a. nonspecific<mechanism-based<affinity tags<$TS^{‡}$ analogs
b. nonspecific<affinity tags<mechanism-based = $TS^{‡}$ analogs
c. nonspecific<$TS^{‡}$ analogs<affinity tags<mechanism-based
d. nonspecific<affinity tags<mechanism-based<$TS^{‡}$ analogs
e. nonspecific<mechanism-based=affinity tags<$TS^{‡}$ analogs

15. The K_M for the conversion of L- to D-Proline in the peptide AEPF by proline isomerase is $1.2x10^{-3}$ M. Under uncompetitive inhibition, the apparent K_M becomes $4.0\ x10^{-4}$ M and the apparent V_{max} is $8.0\ x10^{-6}$ M s^{-1}. The V_{max} for the uninhibited isomerization reaction is:

a. $2.7x10^{-6}$ M s^{-1}
b. $2.0x10^{-2}$ M s^{-1}
c. 0.32 M s^{-1}
d. $2.4x10^{-5}$ M s^{-1}
e. $1.4x10^{-8}$ M s^{-1}

For questions 16 to 18, consider the metabolic pathway show below that converts substrate A to B with the enzyme A-ase, B to C with B-ase(I), and so forth.



16. What is the mechanism of regulation of C-ase?

a. Positive allostery
b. Feedback Inhibition
c. Isoenzymes
d. Reversible Covalent modification
e. Proteolytic Activation

17. What is the mechanism of regulation of B-ase?

a. Positive allostery
b. Feedback Inhibition
c. Isoenzymes
d. Reversible Covalent modification
e. Proteolytic Activation

18. What is the mechanism of regulation of A-ase?

a. Positive allostery
b. Feedback Inhibition
c. Isoenzymes
d. Reversible Covalent modification
e. Proteolytic Activation

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19. Which of the following is *not* a nucleophile?

a. alkoxide
b. carbanion
c. thiolate
d. amine
e. carbonyl

20. During α,β–elimination, the α–proton is acidic and can be abstracted by a basic catalytic residue because:

a. The resulting enolate ion is stabilized through resonance
b. Surrounding active site water can hydrogen bond with the carbonyl
c. The carbonyl carbon is electron-rich
d. The resulting carbanion is more stable than neutral carbon
e. Carbon is more electronegative than oxygen

Short Answer.

21. You perform an experiment to measure the initial rate of reaction for 5 nmol/mL happydase, an enzyme that obeys Michaelis-menten kinetics. From the Lineweaver-Burke plot, you derive a y-intercept of 3.5×10^{-5} $mmol^{-1}$ mL s, and generate the data found in the table below. What is the catalytic efficiency of happydase for its substrate? (**8 marks**).

[S] (μM)	v_o (μmol mL^{-1} s^{-1})
320	169
160	132
80	92
40	57.2
20	32.6
10	17.5

Y-int=1/Vmax, Vmax=28571 mmol mL-1 s-1 (2 marks)
Vmax=kcat$[E]_T$, kcat=5.72×10^9 s-1 (2 marks)
v=(Vmax[S])/(Km+[S]) or slope=Km/Vmax (1 mark)
Km= (some value)μM (1 mark)
Catalytic efficiency=kcat/Km= (some value) mM-1 s-1 (2 marks)

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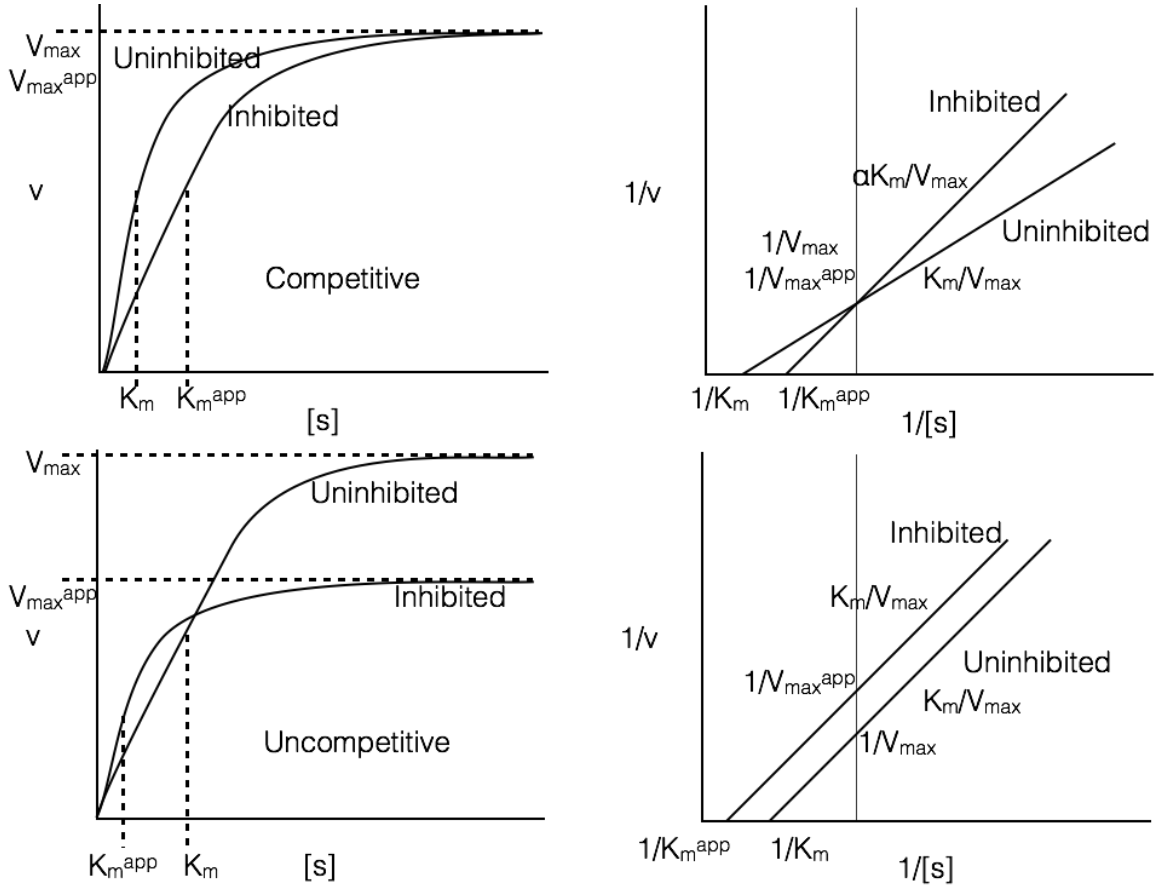
22. You have discovered a small molecule inhibitor of happydase called testostatin. You measure the initial velocity of the enzyme-substrate reaction in the presence of testostatin, and you find a resulting apparent K_M=2.1 μM and apparent V_{max}=9.53 μmol mL^{-1} s^{-1}. Indicate the mechanism of reversible inhibition of happydase by testostatin in the box provided. Draw the Michaelis-Menten and Lineweaver-Burke plots on the axes provided, including a curve expected for the uninhibited reaction and a curve expected for the inhibited reaction. For the Michaelis-Menten plot, label K_M, apparent K_M, V_{max}, and apparent V_{max}. For the Lineweaver-Burke plot, label the x-intercept, y-intercept, and slope in terms of K_M and/or V_{max}. (**14 marks**)

Competitive OR uncompetitive
(depending on answers to Q21)



1 mark for mechanism of inhibition, one for each graph drawn properly, and one mark for each label.

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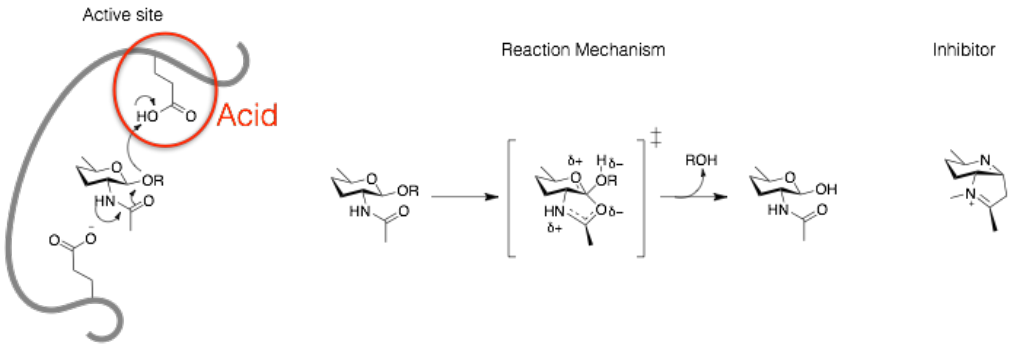
23. Three enzyme-catalyzed reactions are shown below. For each reaction, you are given the active site and catalytic mechanism, the reaction proceeding from substrate to product through the rate-limiting transition state, and an inhibitor of the enzyme.

For all reactions, identify the type of enzyme catalytic mechanisms used in the reaction in the left box provided, and identify the type of irreversible inhibition for the given inhibitor in the box on the right. For mechanisms involving acid/base catalysis, circle the acidic/basic residue(s) directly interacting with the substrate in the active site diagram and label as acidic/basic. Where applicable, circle the cofactor(s) or coenzyme(s) and label each as cofactor or coenzyme. (**24 marks**)

(a)



Catalytic Mechanism(s)

Acid/Base

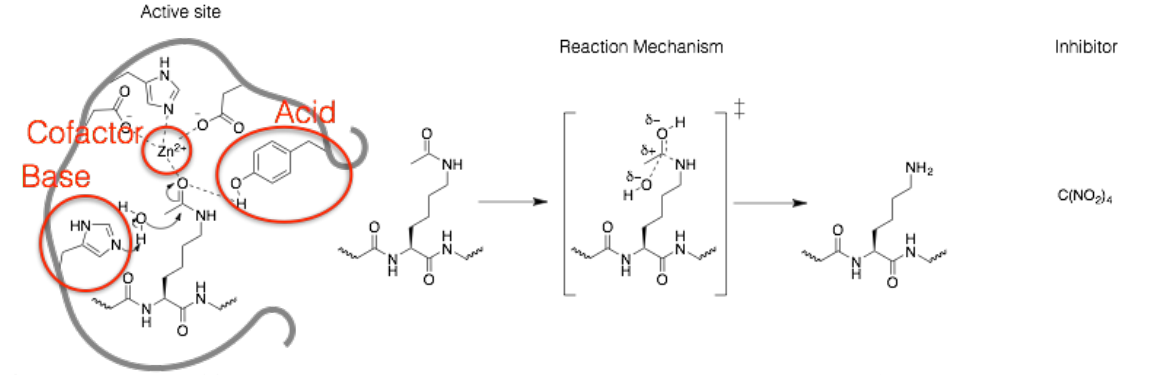
Mechanism of Irreversible Inhibition

TS‡ Analog

4 marks

A

(b)



Catalytic Mechanism(s)

Acid/Base

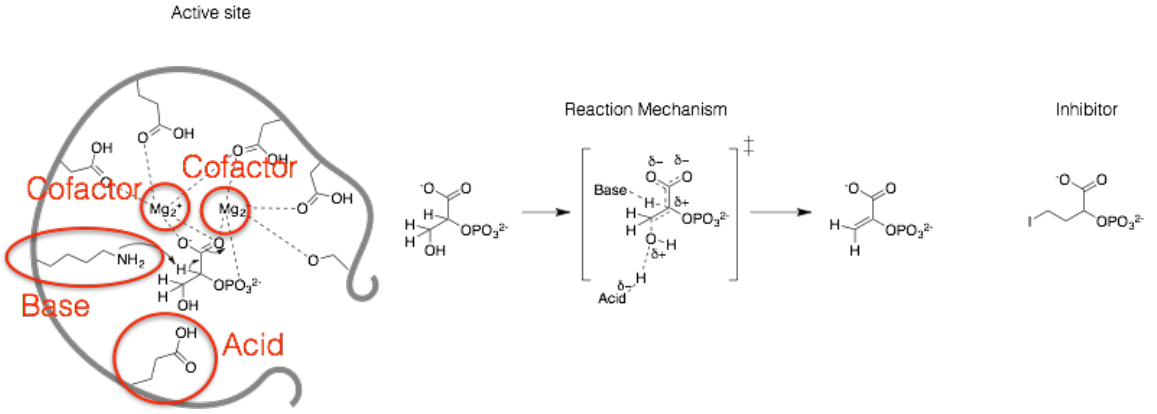
Metal Ion

Mechanism of Irreversible Inhibition

Nonspecific Irreversible

9 marks

(c)



Catalytic Mechanism(s)

Acid/Base

Metal

Mechanism of Irreversible Inhibition

Affinity Label

11 marks