BIOC*3560 S'17 ANSWER KEY

University of Guelph
Department of Molecular and Cellular Biology

BIOC*3560

Mid-term Examination

27 June 2017

Time: 60 minutes

Check your exam paper carefully; you should have 12 different pages, not including the cover sheet on the preceding page. Make sure your **seven-digit student ID number** is entered correctly in **the space provided above** and **on the computer scoring sheet**. **Carefully print** your **name only** on the cover sheet preceding this one.

Answer Part I, questions 1-25, on the computer scoring sheet provided. Only one option is correct for each of these questions. Use **black lead pencil** to fill in the circles corresponding to your answer; erase cleanly if you make a mistake. **Do not use ink or white-out on the computer scoring sheet.**

Answer Part II, questions 1-10, directly on the question paper. **Answer only in ink (NOT red!) and only in the space provided**.

Illegible and otherwise unreadable, incomprehensible or unclear answers & writing **will be considered incorrect**. Answers in pencil will be graded but cannot be regraded.

Part I: MULTIPLE CHOICE QUESTIONS 1- 25 (*25 marks*)

For each question, please fill in the circle corresponding to the correct answer on the computer scoring sheet provided

1. Which statement is **true** with respect to protein-ligand interactions?
   (A) The interactions are not specific.
   (B) The interactions are irreversible.
   (C) The interactions are not regulated.
   (D) The interactions are reversible. ***
   (E) Proteins can bind only 1 ligand.

2. Select the statement that accurately describes the O_2-binding curves of globins.
   (A) The hemoglobin-O_2 binding curve is a straight line.
   (B) Hemoglobin and myoglobin have reciprocal O_2-binding curves.
   (C) Hemoglobin has a sigmoidal O_2-binding curve. ***
   (D) Myoglobin has a sigmoidal O_2-binding curve.
   (E) Hemoglobin and myoglobin have sigmoidal O_2-binding curves.

3. In myoglobin, the iron atom forms coordination bonds with 5 N atoms. 4 of these are within heme. Where is the N atom that forms the 5^{th} coordination bond?
   (A) in the R group of His F8 ***
   (B) in the R group of His HC3
   (C) in α-amino group His F8
   (D) in α-amino group of His HC3
   (E) in α-amino group of His E7

4. Which statement accurately describes molecular events caused by O_2-binding in Hb?
   (A) Small movement of the iron atom within heme causes helix F to shift. ***
   (B) Small movement of helix F breaks ion pairs between helix E and helix F.
   (C) Small movement of helix F disrupts interaction between O_2 and His E7.
   (D) Salt bridges between similar subunits form.
   (E) Salt bridges between unlike subunits form.

5. How does CO_2 influence O_2-binding by hemoglobin?

(A) It binds as a carbamate group, forming salt bridges and increasing affinity for O_2.

(B) It binds as a carbamate group, forming salt bridges that stabilize the T state. ***

(C) It binds to amino acid side chains, decreasing K_d.

(D) It binds to amino acid side chains, increasing K_d.

(E) CO_2 competes with O_2 for binding to the heme group.

6. If O_2-binding by hemoglobin was consistent with a **sequential model** of ligand-binding, then which of the following is predicted to be correct?

(A) O_2-bound subunits in the R state could be next to subunits in the T-state ***

(B) O_2-bound subunits in the R state can never be adjacent to subunits in the T-state.

(C) O_2-bound subunits in the T state would block O_2-binding by subunits in the R-state.

(D) R_2T_2 Hb would contain no O_2.

(E) None of the above.

7. Which of the following statements regarding free heme is true?

(A) It has a higher affinity for O_2 than for CO

(B) It binds oxygen allosterically

(C) It contains 4 pyrrole rings, linked by methane bridges

(D) It contains 4 pyrrole rings, linked by ethane bridges

(E) It contains a system of conjugated C=C bonds ***

8. When studying ligand-binding by proteins, the Hill equation is useful because

(A) $\mathbf{n_H}$ gives the ligand concentration that saturates the binding sites

(B) $\mathbf{n_H}$ gives an indication of the degree of cooperativity in ligand-binding ***

(C) $\mathbf{n_H}$ defines, exactly, the number of ligand-binding sites in the native protein

(D) $\mathbf{n_H}$ defines the affinity of the protein for ligand

(E) $\mathbf{n_H}$ defines the occupancy

9. The residue number HC2 means:

(A) It is the third residue between helix H and the C-terminus***

(B) It is the third residue between helix H and helix C

(C) It is the third residue in helix H

(D) It is the third residue in helix C

(E) It is a hydrocortisone that prevents skin problems caused by biochemistry

10. Which statement is <u>correct</u> about the structure of aspartate transcarbamoylase?

(A) The substrate-binding sites are in between catalytic (c) chains. ***

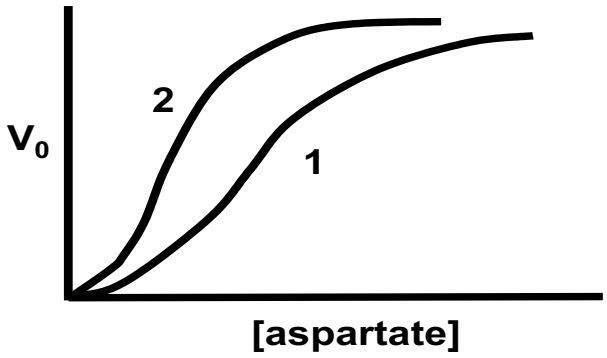
(B) The regulatory subunits are trimers.

(C) The modulator binding sites are at the interfaces of catalytic chains.

(D) The catalytic subunits are dimers.

(E) It contains three trimers connected by two dimers.

Questions 11 and 12 refer to the following diagram, which shows reaction curves for aspartate transcarbamoylase (ATCase) with carbamoylphosphate and different concentrations of aspartate, in the absence (1) and presence (2) of ATP.



11. Which of the following statements is <u>incorrect</u>?

(A) It does not obey Michaelis-Menten kinetics.

(B) Curve 1 has a higher $K_{0.5}$ than curve 2.

(C) Curves 1 and 2 have the same v_{max}.

(D) Curve 2 is hyperbolically shaped. ***

(E) It binds substrate cooperatively.

12. How has ATP affected catalysis by the ATCase enzyme?

(A) ATP has decreased the affinity for substrate.

(B) ATP has decreased the reaction rate.

(C) ATP has increased $K_{0.5}$.

(D) ATP has decreased $K_{0.5}$. ***

(E) ATP has competitively blocked substrate binding.

13. N-(phosphonacetyl)-L-aspartate (PALA) is useful in the study of aspartate transcarbamoylase because:

(A) It is a bisubstrate analog that mimics free substrates.

(B) It is a bisubstrate analog that mimics the reaction products.

(C) It is a bisubstrate analog that mimics a reaction intermediate. **

(D) It 'locks' the enzyme in the T state.

(E) It inhibits the T → R transition.

14. During activation of aspartate transcarbamoylase, conversion from the T to R state involves the following:

(A) movement of catalytic trimers away from each other

(B) breaking a salt bridge between D236c and K143r

(C) forming a salt bridge between D236c and K143r

(D) A and B ***

(E) A and C

15. How does phosphorylation of <u>glycogen phosphorylase</u> affect the conformation of this enzyme?

(A) Serine14-PO_4 disrupts electrostatic interactions, favouring the R state. ***

(B) Serine14-PO_4 disrupts electrostatic interactions, stabilizing the T state.

(C) Serine14-PO_4 forms salt bridges decreasing affinity for substrate.

(D) Serine14-PO_4 forms salt bridges locking the enzyme in the T state.

(E) Serine14-PO_4 electrostatically blocks substrate binding.

16. What type of catalytic activity do caspases have?

(A) They are serine proteases.

(B) They are cysteine proteases. ***

(C) They are serine phosphatases.

(D) They are tyrosine phosphatases.

(E) They are serine/threonine kinases.

17. Which of the following is <u>not likely</u> to contribute to the *regulation* of an enzyme's activity?

(A) Proteolytic cleavage

(B) Removal of a prosthetic group ***

(C) Phosphorylation

(D) Dephosphorylation

(E) Interaction with a regulatory protein

18. Select the statement that accurately describes the effect that cyclin-binding has on CDK-2 structure.

(A) The T-loop moves to block access to the catalytic site.

(B) Glu51 moves toward the active site and access to Thr160 is blocked.

(C) Glu51 moves toward the active site and Thr160 is exposed. ***

(D) The PSTAIRE helix shifts out of the substrate-binding site.

(E) All of the above.

19. Which of the following is true regarding the effect that phosphorylation has on CDK-2 activity.

(A) CDK activity is increased.

(B) The R state of the enzymes is stabilized.

(C) Salt bridges form with Thr160.

(D) Dephosphorylation of Tyr15 is required for substrate-binding.

(E) All of the above. ***

20. Glycogen synthase kinase-3 (GSK-3) can be autoinhibited **and** can also bind to its substrate through the function of the following:

(A) a protein methylation site
(B) an adenylylation site
(C) a phosphoserine-binding domain ***
(D) a phosphotyrosine-binding domain
(E) a phosphohistidine-binding domain

21. Protein sequence analysis reveals that a novel protein contains a Src-homology-2 (SH2) domain. Which of the following of following can this protein bind?

(A) Proteins containing prolines
(B) Proteins containing phosphoserine
(C) Proteins containing phosphotyrosine ***
(D) Proteins containing phosphoproline
(E) Proteins containing methylation

22. The poison warfarin impairs blood clotting because it:

(A) enhances the activity of vitamin K
(B) inhibits vitamin K-dependent activation of platelets
(C) inhibits vitamin K-dependent γ-carboxylation of thrombin
(D) inhibits vitamin K-dependent γ-carboxylation of prothrombin ***
(E) inhibits vitamin K-dependent γ-carboxylation of transglutaminase

23. PKA activation is short lived because:

(A) cAMP is altered by cyclic nucleotide phosphodiesterase ***
(B) cAMP is altered by trypsin
(C) cAMP is altered by chymotrypsin digestion
(D) cAMP is altered by chymotrypsinogen digestion
(E) cAMP is altered by the ceullar pH

24. What could happen to the sequence T-R-K-S-A-L in the presence of PKA?

(A) The Thr (T) would be phosphorylated

(B) The Ser (S) would be phosphorylated

(C) The Ala (A) would be phosphorylated

(D) It would bind but not be phosphorylated ***

(E) It would bind but be phosphorylated at Ser (S) or Thr (T)

25. Assume we have 50% normal Hb or 50% of O_2 binding sites are occupied with CO

(A) Anemia is more severe

(B) CO is more severe ***

(C) Both are more or less equally bad

(D) Neither compares to the damage that this midterm will do to my average

(E) All of the above

BONUS QUESTION

26. Who is the best biochemistry professor ever? Note: There may or may not be more than one correct answer.

(A) False

Do not write in the space below.

Answers written here will not be marked.

<u>Part II</u>: SHORT ANSWER QUESTIONS 1 - 10 (*25 marks*)

Please answer the following questions in the space provided.

1. **(a)** Describe the structural importance of His HC3 in hemoglobin (*3 marks*)

- His HC3 forms 2 salt bridges that stabilize the T state (1)
- one salt bridge is formed with Asp FG1 on the β subunit (1)
- one salt bridges is formed with Lys C5 on the α subunit (1)

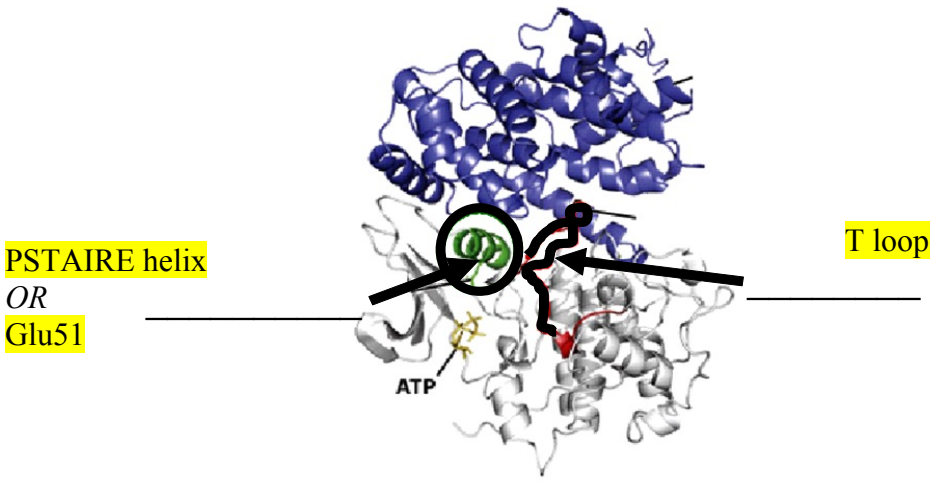
(b) What effect will <u>low</u> pH have on this? Why? (*2 marks*)

- HisHC3 must be protonated (1/2) for salt bridge/ion pair (1/2) with Asp FG1 (1/2)
- stabilizes the T state (1/2) *OR* lower O_2 affinity (1/2)

2. How does binding of 2,3-bisphosphoglycerate (BPG) to hemoglobin alter the structure/function of the protein? (*2 marks*)

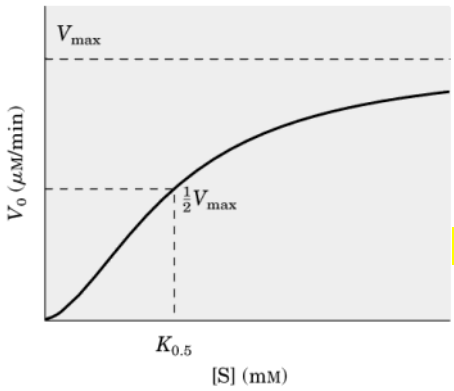
- BPG binds to the central cavity of Hb (1)
- forms salt bridges (1/2) that a) stabilize the T-state (1/2) *OR* b) reduces affinity for O_2 (1/2)

3. Label the two structures indicated in the diagram of CDK below. (*2 marks*)



Part II - cont.

4. (a) Below is an activity curve for the enzyme **midtermase**. On the graph, draw the activity curve you would predict for this enzyme in the presence of increased positive heterotropic allosteric activator (*1 mark*)



← curve must be shifted to left and have sigmoidal shape

5. Explain the molecular mechanism by which CO affinity for heme is 20,000x higher than O_2 with free heme but only 200x higher in hemoglobin. (*2 marks*)

Steric hindrance/clash between His E7 *or* distal His and CO (1)

Favourable hydrogen-bond between O_2 and His E7 (1)

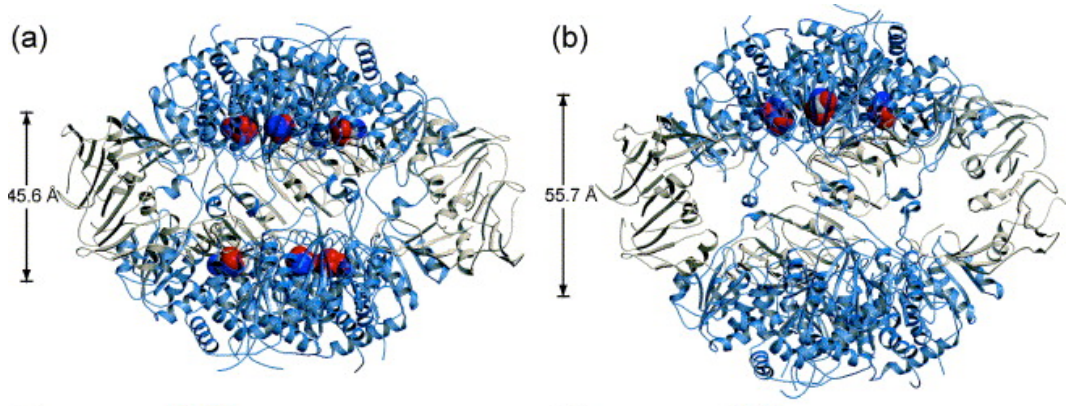
6. Write the letter corresponding to one of the structural/functional features on the left in the space beside the appropriate amino acid in the list on the right. This is complete when amino acid on the right has **one** letter beside it (you will not use every letter on the left). (*4 marks*)

(A) proximal histidine
(B) salt bridge with Arg 50
(C) salt bridge with D236c
(D) "catalytic triad"
(E) salt bridge with Ile16
(F) distal histidine
(G) catalytic site of CDK

_G__ glutamate 51
_F__ H-bond with O_2
_B__ PO_4-Thr160 of CDK
_A__ His F8 of hemoglobin

Part II cont.

7. Figure 2 (a) and (b) of the paper by Stieglitz *et al*. about aspartate transcarbamoylase is shown below.



(a) X-ray structure of the wild type enzyme bound to an analog (PAM) of carbamoyl phosphate.

(b) X-ray structure of the D236Ac mutant enzyme bound to an analog (PAM) of carbamoyl phosphate.

What does a comparison of the structures in (a) and (b) tell us about the D236Ac mutant form of the enzyme? (*2 marks*)

- The D236Ac mutant adopts the R state when bound only to analog of CP (PAM)
- This can occur when only one of the catalytic subunits has PAM bound *OR*
- It follows the concertated model

8. Name **two** of the four effects that phosphorylation can have on a protein (*2 marks*)

Two of:

1) Introduce a sterically bulky group
2) Charge – allowing electrostatic interactions (attraction, repulsion)
3) Oxygen atoms that can participate in hydrogen-bonds
4) Site for protein-protein interactions

Part II cont.

9. (a) What amino acids can protein kinase A phosphorylate? (*1 mark*)

Ser and **Thr**

(b) What other kinds of amino acids can be phosphorylated by kinases other than PKA? (*1 mark*)

Tyr and **His**

10. Fill in the blanks (*3 marks*):

Factor XIIIa is a transglutaminase that is activated by the enzyme thrombin . The role of factor XIIIa is to covalently link fibrin monomers *OR* glutamine & lysine.