Name Key

This exam is schedule for 75 minutes and I anticipate it to take the full time allotted. You are free to leave if you finish. The exam is split into two sections. Part 1 is multiple choice – select the most correct answer in each question. Part 2 is composed of several short answer questions. **For all reaction mechanisms, make sure to regenerate the active site for a complete catalytic cycle.**

Part 1. Clearly circle the most appropriate answer. (3 pts each).

1. Which is not a common type of catalytic strategy?

metal ion electrostatic nucleophilic acid-base covalent

2. Lysozme is an example of a(n)

Oxidoreductase lyase hydrolase Schiff base transferase

3. The catalytic triad in trypsin uses _____ as a nucleophile.

Histidine cysteine aspartic acid serine lysine glutamic acid

4. For ideal enzymes that obeys all assumptions, K_M is best described as the concentration at which _____ of an enzyme is present as an enzyme-substrate complex.

25% 50% 75% 100%

5. k_{cat} is a _____ order rate constant.

0^{th} 1^{st} 2^{nd} 3^{rd}

6. Experimentally determined K_M and V_{max} values change when a **competitive** inhibitor is present.

True False

7. Experimentally determined K_M and V_{max} values change when an **uncompetitive** inhibitor is present.

True False

8. Which of these amino acids is least likely to participate in acid-base catalysis?

Histidine cysteine aspartic acid serine lysine glutamic acid

9. Lysozyme cleaves NAG-NAM glycosidic bonds using only acid-base catalysis.

True False

10. Which of these could drive the oxidation of $Fe^{2+} \rightarrow Fe^{3+}$?

Thiamin FAD NADH PLP Biotin

11. Catalysis is most efficient when the energy of the _____ is minimized.

Enzyme Substrate ES complex Transition State Products

12. Which K_M corresponds to the lowest affinity?

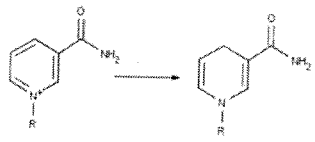
20 mM 20 μM 20 nM 20 pM

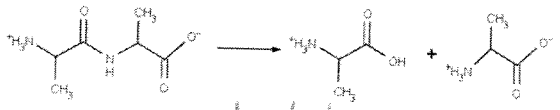
Part 2 – Not so short answer.

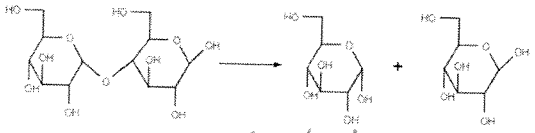
13. How can metal ions promote catalysis? (3 pts)

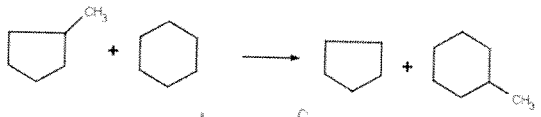
redox
electrostatic stabilization
substrate orientation

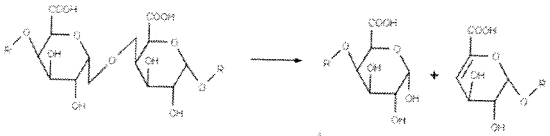
14. For each of the following reactions, indicate what class of enzyme is required to catalyze the reaction. Note that the reactions are not necessarily balanced (1 pt each)

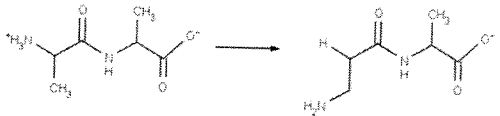
a.  oxidoreductase

b.  hydrolase

c.  hydrolase

d.  transferase

e.  lyase

f.  isomerase

15. Explain the Bohr Effect. Include all relevant equilibria. (5 pts)

① $CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$

② $Hb + O_2 \rightleftharpoons HbO_2 + H^+$

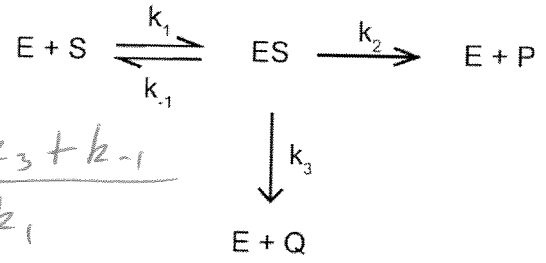
pH changes can facilitate O_2 release from Hb. CO_2 is a product of respiration in muscle tissue. This CO_2 is converted to HCO_3^- & H^+ according to equation 1. This occurs in the capillaries. This acidification results in extra release of O_2 from Hb according to ②

16. Draw the **complete** kinetic scheme describing enzyme kinetics. Make sure to indicate which steps are reversible and irreversible. Show which (if any) of the steps can be approximated as a single kinetic event. Explain why this approximation can be made. (8 pts)

$$E + S \underset{(1)}{\overset{K_1}{\rightleftharpoons}} ES \underset{(2)}{\overset{K_2}{\rightleftharpoons}} TS^{*} \underset{(3)}{\overset{k_3}{\longrightarrow}} EP \underset{(4)}{\overset{K_4}{\rightleftharpoons}} E + P$$

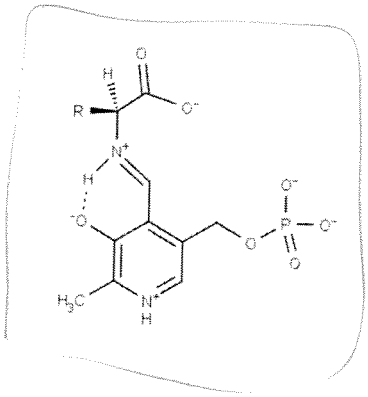
events 1, 2 and 4 are all reversible equilibria describing formation of the enzyme-substrate complex, conversion to the transition state and release of product, 3 is an irreversible kinetic step that describes the conversion of substrate in the TS to products. This is considered irreversible b/c the Ea to get back to the TS will be very large. 2 → 4 can be condensed to a single event b/c once the TS is formed, the reaction is assumed irreversible

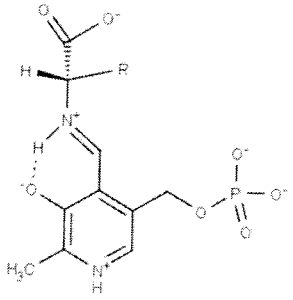
17. Consider the reaction scheme below where 2 different types of products can be formed. Without deriving anything, what is K_M with respect to the rate constants? (5 pts)

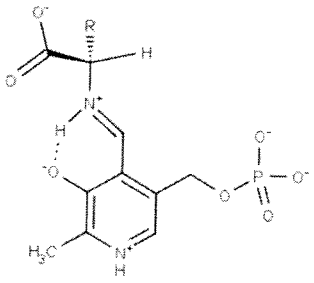


$$K_M = \frac{k_2 + k_3 + k_{-1}}{k_1}$$

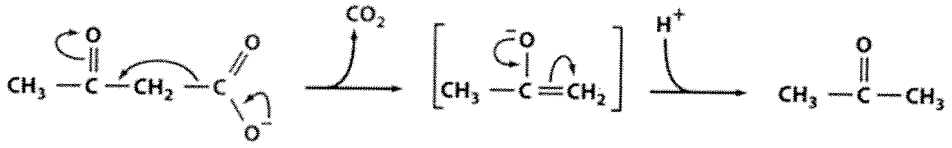
18. Circle the structure that is most likely to result in decarboxylation of the substrate. (2 pts)



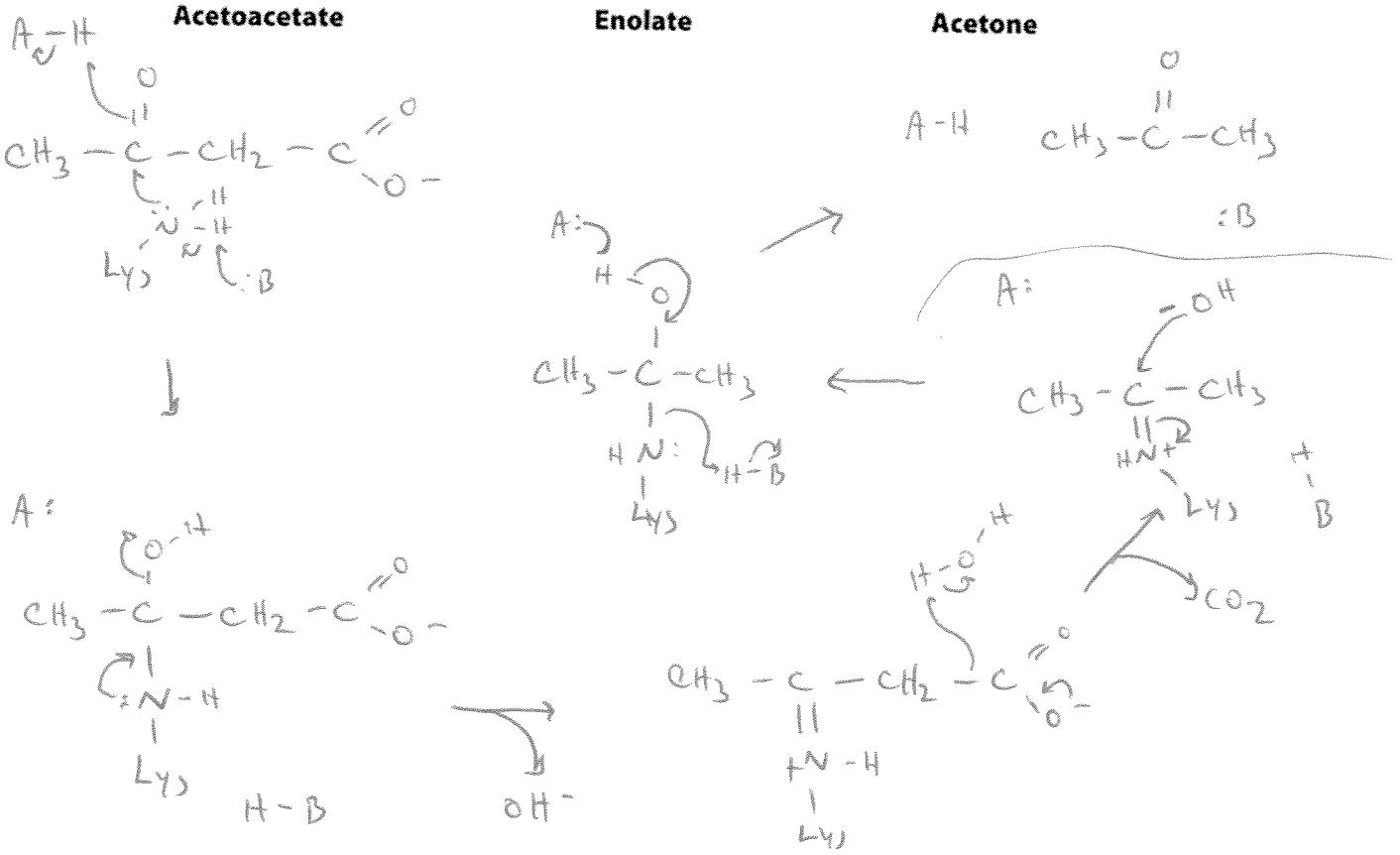




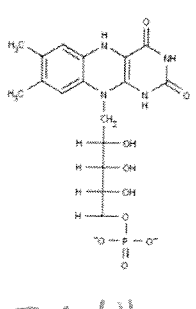
19. The reaction below proceeds very slowly due to the formation of the enolate intermediate. Biological systems often use Schiff bases to bypass this unstable transition state. Propose a mechanism for this catalyzed reaction. Show all steps. (10 pts)



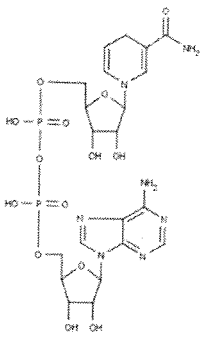
Acetoacetate **Enolate** **Acetone**



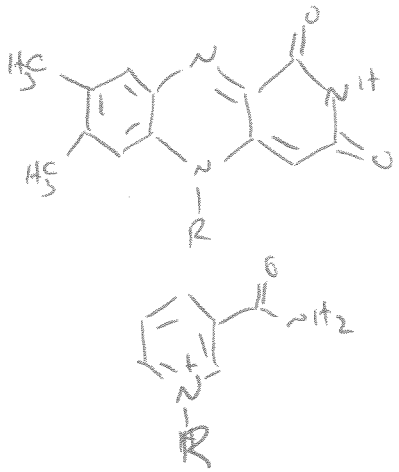
20. Shown are the reduced forms of two cofactors we discussed. Name them and draw the oxidized form. Feel free to use 'R' for any region not involved in redox. (2 pts)



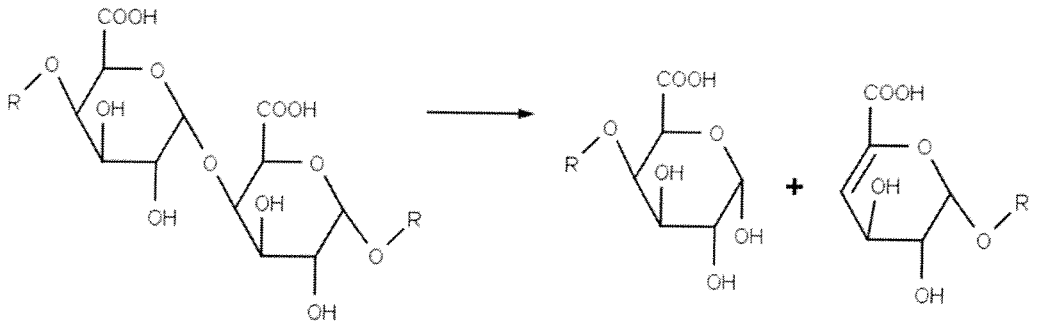
$FMNH_2$

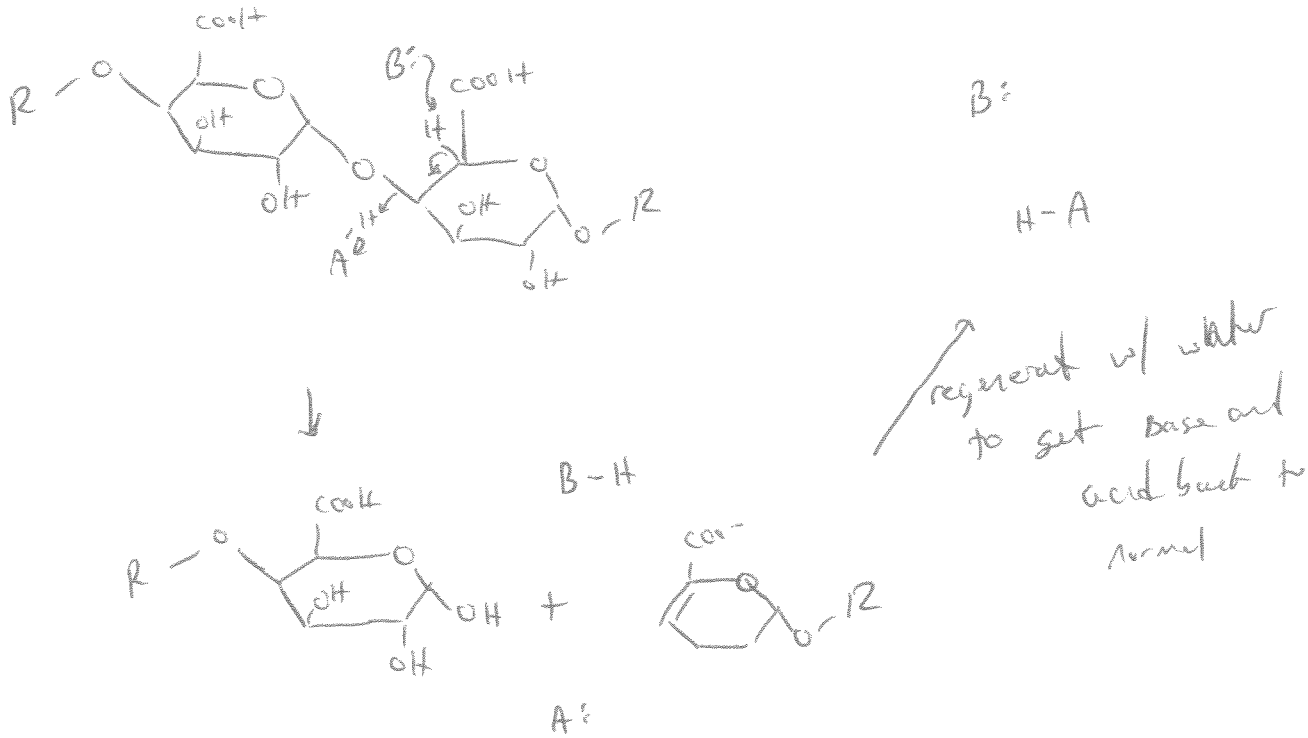


NADH



21. Pectate Lyase catalyzes the β-eliminative cleavage of polygalacturonic acid. Propose a mechanism. (8 pts)

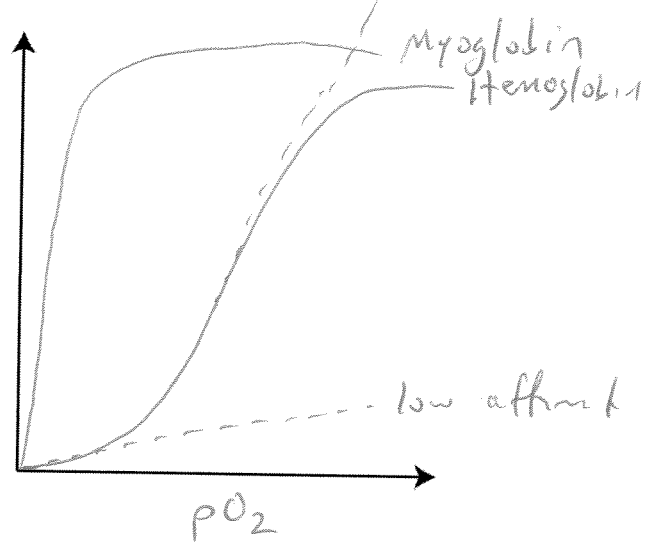




22. Allosteric proteins.
    a. What is an allosteric protein (2 pts)

a protein that has at least 2 binding sites → one is the active site, one is the allostric site. when a molecule binds at the allostric site (which is spacially distinct from the active site) it influences the activity / affinity of the active site

b. On the graph, sketch the O_2 binding curve for myoglobin and hemoglobin. Make sure to label the axes. Indicate the regions of high and low O_2 affinity where applicable. (3 pts)



c. Why are these shapes different? Be specific. (2 pts)

myoglobin only binds a single O_2 with high affinity. hemoglobin binds 4 O_2 and binding of O_2 increases the affinity for subsequent O_2 binding events

d. Other than hemoglobin, describe one example of how an allosteric response can be important in biological systems. (3 pts)

propose one of many. We talked about transcriptional regulators. The lac repressor binds tightly to DNA until lactose or IPTG bind to it. This influences the affinity for DNA

e. Describe the similarities and differences between the concerted and sequential models of allostery. Include a sketch if you like. (5 pts)

Both are models that describe allosteric communication in protein.

Similarities

binding @ one site influences affinity/activity
must have a structural change to trigger response
binding event triggers structural change
allosteric proteins are multidomain proteins (multi subunit)

differences

the concerted model is an all or none event. If one subunit change shape, they all do. The sequential model is not restricted to this. Adjacent subunits change structure, not necessarily all of the protein