Questions 1-5: True or False? (2 points each)

1. The reaction pathway of a catalyzed reaction must be the same as that of the uncatalyzed reaction.
2. In the symmetry model of cooperative ligand binding, the binding of substrate changes the equilibrium between high- and low-affinity states of the protein.
3. Apomyoglobin cannot bind oxygen.
4. The heme iron is oxidized from Fe(II) to Fe(III) when hemoglobin binds oxygen.
5. Bisubstrate reactions are common in biochemistry.

6. (4) By definition, an allosteric protein (choose all that apply):
   a. Has more than one binding site
   b. Binds multiple, different ligands
   c. Undergoes a conformational change in response to binding of an effector
   d. Shows cooperativity in ligand binding

7. (3) Choose the best explanation for why cooperativity in binding oxygen is important to the function of hemoglobin:
   a. It allows hemoglobin to bind more oxygen at the lungs
   b. It allows for more efficient transfer of oxygen
   c. It allows for adaptation to different altitudes
   d. It allows hemoglobin to be sensitive to pH

8. (4) Histidine is an important amino acid in the structure and function of hemoglobin. Briefly list up to four specific ways that histidine contributes to the function of hemoglobin. (Two correct answers will earn you full credit; additional correct answers will earn extra credit; wrong answers will cause you to lose points.)

9. (3) Briefly describe the function of myoglobin. (10 words or fewer)

10. (4) Is the p50 of O2 binding by myoglobin (Mb) higher than, lower than, or equal to that of hemoglobin (Hb)? Why is this important? (Explain in 30 words or fewer.)
Questions 11-16: True or False? (2 points each)

11. In the SN1 mechanism proposed for lysozyme, the enzyme forms a covalent bond with the substrate.
12. The reaction catalyzed by lysozyme can be considered a group transfer reaction.
13. Lysozyme is a glycosidase.
14. Chymotrypsin catalyzes a ping-pong (double-displacement) reaction.
15. The so-called “oxyanion hole” is a feature of all serine proteases.
16. For a particular enzyme, a transition state analog will likely have a higher Km than a substrate analog.

17. (5) Which of the following pieces of evidence would indicate that two enzymes are related by divergent (as opposed to convergent) evolution? Choose all that apply.
   a. They have similar sequences
   b. They have very different sequences
   c. They have similar active sites
   d. They have similar structures
   e. They have different structures
   f. They have similar functions
   g. The positions of active-site residues in the sequence are similar
   h. They act on the same substrate

18. (10) In addition to amide bonds, chymotrypsin is able to hydrolyse ester bonds, such as the one in p-nitrophenylacetate, shown below:

   \[
   \text{H}_3\text{C}\text{O} - \text{O} - \text{C} - \text{O} - \text{NO}_2
   \]

   a. Starting with the active-site framework provided, draw the first tetrahedral intermediate formed in the hydrolysis of p-nitrophenylacetate \textit{and how it is bound by the enzyme}. Be sure to complete the provided functional groups by adding any missing bonds, atoms, charges, etc. Indicate any H-bonds with dotted lines.

   b. Based on your drawing, would you expect the catalytic efficiency to be greater for the hydrolysis of p-nitrophenylacetate or for chymotrypsin’s biological substrate (that is, a peptide)? Why? Explain in 50 words or fewer.
19. (2) The reaction $X \rightarrow Y + Z$ is:
   a. Unimolecular
   b. Bimolecular
   c. Termolecular

Questions 20-23 relate to the following reaction:
\[ A \xrightleftharpoons[k_4]{k_3} B + C \quad K_{eq} = 100 \text{ M} \]

20. (3) Write an expression for the rate (velocity) of this reaction (using rate constants and concentrations).

21. (2) What are the units of $k_4$?

22. (2) What class of enzyme would most likely catalyze this reaction?

23. (5) If $[B] = 100 \text{ mM}$, what relationship between $[A]$ and $[C]$ must exist for this reaction to be spontaneous in the forward direction? Show your work.

Questions 24-26 relate to the enzyme-catalyzed reaction, which proceeds via the following steps:
\[ E + A \xrightleftharpoons[k_1]{k_2} EA \xrightarrow[k_2]{k_1} E + B + C \]

24. (5) Would using this reaction to derive the rate equation (versus using the reaction we discussed in class) give the same result? In other words, would starting with this reaction allow one to derive the Michaelis-Menten equation? If so, why don’t the differences between the two reactions matter? If not, what in the derivation would change, and what equation would result? (Explain in 50 words or fewer.)

25. (3) Write an equation (using rate constants and concentrations) for $E$ at steady state.

26. (2) True or false? When $E$ is at steady state, $EA$ is also at steady state.
(For questions 27-30:) In anaerobic exercise, muscle cells are not able to get sufficient oxygen to allow for the complete oxidation of glucose to CO$_2$. Instead, glucose is broken down and converted to lactate through the pathway shown. (For clarity, side reactants and products have been shown for the forward direction only.)
27. (4) Name the class of enzyme that catalyzes each of the following steps: 6, 7, 8, 9.

28. (7) Enzyme 4, aldolase, catalyzes the breaking of the 6-carbon chain into two 3-carbon compounds. The first portion of a possible mechanism for aldolase is shown below. For each amino acid depicted, list the catalytic mechanism(s) in which it participates.

29. (5) The mechanism of aldolase (above) involves the formation of a Schiff base. How does the formation of this intermediate help in catalyzing the breaking of the C3-C4 bond of fructose-1,6-bisphosphate (the substrate)? (Explain in 40 words or fewer.)

30. (5) Explain how the excess protons produced during anaerobic exercise promote the delivery of oxygen to muscle tissue. (60 words or fewer)