It’s Enzyme Time (12 points)

You overhear a student say the following quote:

“…it’s the kcat that’s important! If you know the kcat of an enzyme, you can predict what the maximum rate of the enzyme will be, and what the 50% saturation substrate concentration will be as well…”

Let’s think about this quote:

1) (1pt) Define the kcat for an enzyme:

2) (2pt) Write the version of the Michaelis-Menton equation that specifically includes the kcat (use space to right)

3) (1pt) For an enzyme that follows the Michaelis-Menton relationship, what is the substrate concentration that will cause 50% of the maximal velocity of the enzyme? (use space to right)

4) (1pt) So, is the student’s quote correct, partially correct or totally wrong, and why (one sentence)?

5) (2pt) Consider an enzyme-catalyzed reaction. Suppose that the uncatalyzed reaction has an activation energy that is higher by 8.7kJ per mole in the absence of enzyme. What letter best describes the effect on the reaction caused by the presence of the enzyme?

   your choice: ___ ___

   A) faster by a factor of \( (8,700/RT) \)  B) faster by a factor of \( e^{(8,700/RT)} \)

   C) faster by a factor of \( e^{-(8,700/RT)} \)  D) effect depends on the original Eact
6) (4pt) Suppose the forward reaction going from S to P has an actual $\Delta G'$ of -33 kJ/mole. Draw an energy diagram using the graph to the right, showing the positions of the S and P, and the effect of the enzyme on the free energy diagram. Indicate the distance on the graph that corresponds to $\Delta G'$. Label the axes please.

7) (1pt) What is the mathematical expression for the ratio of the forward and reverse rate constants for the specific reaction we are discussing above? Choose the best answer from the letter options below:

Letter answer: ______

A) $\frac{\text{k}_f}{\text{k}_r} = e^{\frac{-33,000}{RT}}$

B) $\frac{\text{k}_f}{\text{k}_r} = \frac{33,000}{RT}$

C) $\frac{\text{k}_f}{\text{k}_r} = e^{\frac{33,000}{RT}}$

D) $\frac{\text{k}_f}{\text{k}_r} = (33,000/RT)$

E) $\frac{\text{k}_f}{\text{k}_r} = e^{\frac{\Delta G^\ddagger}{RT}}$

F) $\frac{\text{k}_f}{\text{k}_r} = e^{\frac{(\Delta G^\ddagger + 33,000)}{RT}}$
Graphs and Corruption (10 points)

In class we discussed a Lineweaver-Burke plot as a way to plot enzyme kinetic data. In the spaces that are provided, describe this sort of plot.

8) (1pt) The points on the X (horizontal) axis are:

9) (1pt) The points on the Y (vertical) axis are:

10) (1pt) The X intercept is equal to:

11) (1pt) The Y intercept is equal to:

12) (1pt) Why do people use the Lineweaver Burke plot in analysis of enzyme kinetics (One sentence)

Allosteric enzymes are often encountered in the study of metabolic regulation. Below is a simple rate plot, in which initial enzyme rate is plotted against substrate concentration $S$. You will draw a few curves.

Please label each curve.

13) (1pt) Draw a curve for a cooperative enzyme. Include an approximate $V_{\text{max}}$ for use below.

14) (1pt) Draw a new curve for this enzyme when a heterotropic allosteric activator is added, and label it A.

15) (2pt) Draw a third curve for E when a covalent inhibitor irreversibly destroys half of the enzyme activity, label it C, and indicate on the graph the effect on $V_{\text{max}}$.

16) (1pt) (I ask this every year, because I feel its my responsibility as a professor) What does this kinetic behavior indicate about the structure of this particular enzyme?
Redox Redux (14 points)

Here is a list of standard half-cell potentials for a number of reactions.

1) aKG + CO₂ +2H⁺ +2e- <-> isocitrate = -0.33 V
2) NAD+ + H⁺ + 2e- <-> NADH = -0.32 V
3) FAD + 2H⁺ + 2e- <-> FADH₂ = -0.22 V
4) FMN + 2H⁺ + 2e- <-> FMNH₂ = -0.12 V
5) CoQ + 2H⁺ + 2e- <-> CoQH₂ = +0.10 V
6) Cyt c (Fe³⁺) + e- <-> Cyt c (Fe²⁺) = +0.22 V
7) Cyt a (Fe³⁺) + e- <-> Cyt a (Fe²⁺) = +0.29 V

17) (3pt) These are also known as the E° for the reactions. What does the “prime o” after the E mean?

18) (2pt) Rewrite half-reaction #1 with structures

19) (1pt) What enzyme catalyzes the metabolic reaction that includes half reaction #1?: ________________________________

20) (1pt) Of all the molecules on this table, which one is the best oxidizing agent ________________

21) (4pt) Write a balanced complete reaction combining half-cell reactions #3 and #5 in the direction that would be spontaneous at standard conditions. Include the E° value for the complete reaction

22) (2pt) Set up the equation to compute ΔG° for the spontaneous complete reaction you wrote in 21. You can leave constants as constants.

23) (1pt) What information would you need to calculate the ΔG’ for this reaction in the cell at 25 °C?
You Say Acetate-o and I Say Acetat-o... (12 points)

Bacteria can live on acetate as the sole carbon source. That means that they can use the carbon in acetate to make every carbon compound they need. But first, they have to get acetate into the appropriate metabolic pathway to use those carbons.

24) (1pt) What is the general name for the large family of pathways that allow small molecules to be used to make bigger, fancier molecules?

____________________________

Here is the reaction that bacteria use to get acetate into metabolism. It is catalyzed by an enzyme called acetyl thiokinase:

\[ \text{CH}_3\text{-CO}_2^- + \text{HS-CoA} + \text{ATP} \rightarrow \text{CH}_3\text{-CO-S-CoA} + \text{AMP} + \text{PiPi} \]

25) (1pt) What is the name of the acetate-containing product? Write it out in “English” __________________________________________________________

26) (1pt) What role do you think the ATP plays in this reaction (one sentence)?

27) (2pt) Like in many reactions, the acetate forms a covalent intermediate that allows the CH\_3\text{-CO-S-CoA} to form spontaneously. Based on the products formed in this reaction, propose a structure for that intermediate. Write out the structure of the acetate; anything else can be abbreviated with letters, in the box provided. This is a picture of a single molecule. (Hint: think good leaving group…)

28) (1pt) What biochemical pathway do bacteria need to perform net synthesis of carbon compounds from acetate?:

29) (2pt) What two enzymes are unique to this pathway? Just the names please, no reactions.

30) (4pt) Write the reaction for the enzyme in 29 that acts second. Include structures and names of substrate(s) and product(s). Cofactors and carriers, if needed, can be represented by letters, as always.
An Inherited Fumarase Deficiency (14 points)

Sometimes a patient will present with clinical symptoms due to a mutation, referred to as an inborn error, in the gene for a single metabolic enzyme. We have talked about such things, and I will now describe another actual example. It turns out that patients can be born with a complete lack of the enzyme fumarase (Zinn et al. (1987) NEJM 315: 469-75). Not surprisingly, this syndrome is rare and recessive. In the case described, the affected individual had almost no detectable fumarase activity, and died at age 8 months.

31) (3pt) Write out the balanced reaction that fumarase catalyzes. Name and draw the structures of the products and reactants. No cofactor structures necessary.

32) (1pt) Is the resulting product optically active, prochiral, or neither. Put X on the best choice

- optically active ________
- prochiral ________
- neither ________

33) (1pt) What metabolic pathway is this reaction part of?

34) (1pt) In what cellular compartment does this metabolic pathway occur?

35) (2pt) In the report cited above, the authors note that levels of succinate are elevated. Offer a reason why this might be, and feel free to use a reaction or a picture if it helps clarify your explanation.
An Inherited Fumarase Deficiency (cont’)

In order to understand this lethal syndrome, a pathologist is studying a human cell line with the same complete absence of fumarase. He comes to you with several observations that he, not having metabolic biochemistry (he went to Harvard, which has stopped teaching this to undergraduates), does not understand. Below are a couple of his observations that baffle him. Try to help this poorly trained researcher (in all cases the answer is simple, not complex… please don’t freak out).

36) (1pt) The mutant cells have a much higher rate of glycolysis than normal cells. Why would this be? (One sentence)

37) (2pt) The cell line secretes more lactic acid than an identical cell line with normal fumarase activity. Why would this be? (One sentence)

38) (3pt) When a lysate of the cells is tested for biochemical activity, the pathologist discovers that adding phosphoenolpyruvate (PEP) causes the production of malate, which is quite surprising to him. You, however, going to UCSD, are not surprised. You jokingly ask him if he has ever heard of the disease “anaplerosis”, trying to give him a hint. He still doesn’t get it, (he’s really poorly trained), so you simply explain what is going on. Say why this isn’t so surprising and write the relevant reaction, with metabolite structures and the name of the enzyme and cofactor(s). Remember, this is a human cell line.
As American as Apple Pyruvate (16 points)

The production of pyruvic acid is a central feature of metabolism in all organisms. Its synthesis regulation and utilization are all important.

39) (1pt) Draw the structure of pyruvic acid in the box

In fermentation, pyruvic acid is converted into other things. We have discussed two distinct versions of fermentation. One happens in many organisms including animals such as coelacanths, alligators and numerous human tissues. The other type occurs only in microorganisms.

40) (2pt) What is the common function of these two distinct fermentative pathways?

41) (2pt) Write the reaction for conversion of pyruvate by fermentative metabolism that occurs in many animal and human tissues. Include carriers or cofactors, enzyme name(s), and draw the structures of substrates and products.

42) (3pt) Write the reactions for the version of pyruvate fermentation that is specific for microorganisms. Include cofactors, and enzyme name(s), and structures of substrates and products.

As a hint, here is the first verse of entertainer Don Ho’s classic song “Tiny Bubbles”

Tiny bubbles (tiny bubbles)
In the wine (in the wine)
Make me happy (make me happy)
Make me feel fine (make me feel fine) by Loen Pober
As American as Apple Pyruvate (cont’)

When fermentation is not occurring, pyruvate has a very different fate. It is used for subsequent oxidative metabolism, and is made ready for metabolism by the pyruvate dehydrogenase complex (PDH).

43) (2pt) Where are the products of the PDH reaction?

44) (3pt) Below is a list of molecules. Assign the correct number to best indicate their order of involvement in the action of the PDH complex. You do not need to use any number twice. **For any molecule not involved, put an X**. The numbers 1, 4 and an X have already been added, because I’m a nice guy, for a geek.

Red. Lipoic acid_________ Ox Lipoic Acid_1_ FADH2 _4_ NAD+_________

Biotin _________ NADP_______ 2,3,DPG _X_ NADH _________

CoA-SH ___________

There is a cofactor conspicuously missing from this list that is involved in the PDH reaction. In fact, its “business end” has the indicated structure.

45) (2pt) Name the cofactor that this is from and draw a structure showing the covalent adduct formed between this business end and pyruvate.

46) (1pt) What other enzyme complex is very similar to PDH?
Getting into Glycolysis (9 points)

The following carbohydrates can all be recruited for metabolism by glycolysis. For each carbohydrate, match up the most appropriate phrase with the correct sugar by using the capital letter. Some of the phrases are not useful. One letter per sugar. Letters can be used once, more than once, or not at all (1pt each).

47) Glycogen _______  48) Mannose_______  49) Fructose_______

50) Galactose______  51) Glucose________

A) Is broken down into individual glucose-1-phosphate subunits by addition of phosphate  
B) Is attached to a carrier molecule and then directly metabolized by glycolytic enzymes  
C) Can be recruited into metabolism by two similar but distinct pathways.  
D) Is broken down into individual glucose subunits by hydrolysis  
E) Is phosphorylated and then isomerized into a glycolytic pathway molecule  
F) Is broken down into individual glucose 6 phosphate subunits by hydrolysis  
G) Is attached to a carrier molecule and then structurally altered by an enzyme  
H) Is phosphorylated first at the 6 carbon

There are circumstances in which glyceraldehyde is generated during metabolism. Like you know, it is closely related to a glycolytic pathway molecule.

52) (1pt) Draw the structure of glyceraldehyde in the box.  

53) (2pt) What is the most direct route to get this carbon skeleton into the glycolytic pathway? Propose a reaction, and name the product. This is not something you memorized. It is something you can figure out. Use reactant(s) that will insure spontaneity, from what you know about metabolic biochemistry

54) (1pt) Suppose that our old friend 3-phosphoglycerate had a $^{14}$C label at the 2 carbon. Draw the structure of the succinate that would first be generated from metabolism of this labeled 3PG after entering the Krebs cycle
The True Will Set You Free (13 points)

Write a capital T if the statement is true and an F if... you know the drill

55) _______ Ribose is normally made from pyruvate

56) _______ All spontaneous reactions have a negative ΔH

57) _______ NADPH is produced by the pentose phosphate pathway

58) _______ Hexokinase is nearly always functioning at maximum velocity

59) _______ Ubiquinone has one reduced form and one oxidized form

60) _______ The Krebs cycle does not function in anaerobic (no O₂) conditions

61) _______ High levels of NADH slow the Krebs cycle

62) _______ Cytochromes have heme groups that in which iron and sulfur form a complex

63) _______ Glucose is an allosteric regulator of glycogen phosphorylase kinase

64) _______ Glucose is an allosteric regulator of glycogen breakdown

65) _______ The inner membrane of the mitochondrion is more permeable than the outer membrane

66) _______ Acetyl CoA inhibits the pyruvate dehydrogenase complex

67) _______ Governor-Elect Schwarzenegger is planning to have a butt-kicking Green Laser in every UC classroom