Protein preamble (9 points)

Look, another license plate! I made this one on the CA DMV web site, that lets you see what an idea for a vanity plate looks like. Think I'll pass on this one. But just like I promised, here are a few questions on the one letter code and amino acid properties.

1 (2pts) Write the sequence of the peptide spelled out in the plate, using the three letter code (eg. a tripeptide of tryptophan would be TrpTrpTrp).

2) How many of the R groups in the above peptide have a nitrogen molecule? __________

3) How many atoms of sulfur are there in this particular peptide? __________

4) One of the amino acids in the plate above is also found in the chymotrypsin catalytic triad we have discussed. Which one is it __________

5) Briefly describe the function of that amino acid in the catalytic triad (one sentence)

6) Serine (S) is another catalytic triad amino acid. What is the function of serine in the catalytic triad (one sentence)

7 (2pts) Which amino acid on the license plate is most similar to serine, and why?
Enzyme kinetics 101 (or maybe 102…) (13 points)

You study an enzyme in the laboratory. By your work, you find out that this enzyme obeys Michaelis-Menton kinetics, and so two constants, which you call A and B, describe the enzyme's behavior at all substrate concentrations, in the equation shown:

\[ V = \frac{B[E]_{\text{tot}}S}{A + S} \]

8) What is the name of curve that describes your enzymes rate as a function of substrate?

9 (2pts)) Usually the constants in your rate equation have more common labels. What are they?

A is known as __________________

B is known as  __________________

10) What is the concentration of substrate at which your enzyme is operating at 50% of its maximum? (expressed in terms of one of the constants above).

________________

11) What is the concentration of substrate at which your enzyme is operating at 90% of its maximum? (expressed in terms of one of the constants above)

________________

12 (2pts)) What is the physical meaning of B in terms of the enzyme's function?

13 (4pts)) Use the axes to the right to draw a Lineweaver-Burke plot for this enzyme. Label the axes, and the two intercepts. You don't need to say what the slope is.

14) You isolate a variant of your enzyme from another tissue, that catalyzes the same reaction, but it no longer shows Michaelis-Menton behavior. Instead, it show cooperativity. Sketch (no need for labels) what the S vs. V plot on the axes provided (with the V and S) below.

15) What is one structure feature of your new cooperative enzyme that is indicated by its rate behavior?
An ATP-driven reaction: big man, little dog… (8 points)

The reaction shows an ester being formed from a carboxylic acid and an alcohol, from your organic chemistry days. Formation of this ester is energetically unfavorable; it will not happen without added energy. However, when this reaction is coupled to ATP hydrolysis, the reaction occurs spontaneously, and is catalyzed by an enzyme in the cell. The entire coupled reaction goes like this

\[
\text{R}_1\text{-C-}\text{O}^- + \text{R}_2\text{-OH} + \text{ATP} \rightarrow \text{R}_1\text{-C-}\text{O-R}_2 + \text{ADP} + \text{P}_i
\]

We are going to explore how ATP provides the energy to form this ester. It is just like several other examples we have discussed in class. It turns out that an intermediate molecule is formed that has, you guessed it, a good leaving group, allowing the esterification reaction to occur spontaneously.

16 (2pts) Draw that intermediate molecule in the space provided. Wait! Look at the products of the reaction above to decide what your good leaving group must be. Then, think about which of the two organic reactants (not the ATP) would be the nucleophile that will bump off the good leaving group. Then draw your intermediate molecule.

17) Suppose in these conditions the ester formation reaction has a $\Delta G$ of 26 kJ/mole, and the ATP hydrolysis has a $\Delta G$ of -56 kJ/mole. What is $\Delta G$ for the coupled reaction written above? ________________

18) What is the $\Delta G$ for the simple hydrolysis of the ester (back into the carboxylic acid and alcohol) without ATP in these conditions? ________________

19) An enzyme is needed for the reaction to occur as well. Does the enzyme affect the $\Delta G$ of the coupled reaction? Yes or no, and why.

20 (3pts) Sketch an energy diagram for the coupled reaction, with and without enzyme. Label axes, indicate the $\Delta G$ for the reaction, the activation energy $\Delta G^\ddagger$, and the effect of the enzyme. You can use the abbreviations below to make the drawing less cluttered.

\[
\text{R}_1 + \text{R}_2 + \text{ATP} \rightarrow \text{P} + \text{ADP} + \text{Pi}
\]
An inborn error in pyruvate dehydrogenase (11 points)

The pyruvate dehydrogenase (PDH) complex is a key enzyme complex that connects glycolysis to subsequent oxidation of glucose. A number of cases have been reported where a patient has a deficiency in PDH activity due to a genetic loss of one or more activities of the complex. Understandably, the symptoms of PDH deficiency (PDHD) are severe, often resulting in infant mortality, or survival to adulthood with extreme neurological symptoms.

21 (2pts) Write the balanced reaction for what PDH does. You do not need to include cofactors (we'll get to them below), just all reactants and products. No structures needed here.

22 (2pts) The four cofactors NAD, FAD, TPP, lipoic acid all function in PDH catalysis. Write the order of involvement of the cofactors in the PDH reaction as you wrote it above. Just the order, nothing fancy. Like this: NAD, FAD, TPP, lipoic acid. Or whatever.

ORDER:

23 (3pts) Which of the four PDH cofactors are represented below? Label each, and use "none" if the picture is not associated with any of them.

24) Patients with a severe PDH deficiency have limited ways to make ATP from glucose. How do they make most of their ATP from glucose?

25 (3pts) Patients with severe PDHD have elevated blood lactic acid. State why this is the case (one sentence). Write the reaction that forms lactic acid. Name the enzyme and write the reaction, including structures of the three-carbon reactants and products.

a) your sentence:

b) your reaction:
Thinking about drinking (6 points)

This is a blast from the past! This question was on the midterm last year, and I like it so much that I have replayed it here.

Ingested ethanol is detoxified by the liver to harmless acetate. This occurs by a two reaction pathway, in which ethanol is converted into acetaldehyde, and then acetaldehyde is converted into acetate. A two-enzyme pathway. You have never seen this pathway, but your current knowledge will allow you think about ethanol detoxification in clear, chemical terms. Let’s go!

26 (1.5 pts)) First, draw the structures of the three molecules. I have included ethanol. That level of detail is fine.

ethanol  acetaldehyde  acetate

The first step (ethanol to acetaldehyde) is catalyzed by alcohol dehydrogenase. The second step (acetaldehyde to acetate) is catalyzed by aldehyde dehydrogenase. Both use NAD+ as a substrate.

27) Write the reaction for the first enzyme, alcohol dehydrogenase, using structures, and including NAD+ in the reaction.

28) When a person on Antabuse drinks ethanol, what molecule do you expect to accumulate? __________________

29 (1.5 pts)) The \( E^\circ \) for the acetaldehyde/ethanol half reaction is \( -0.197 \text{ eV} \). Write the balanced half-cell reaction, including electrons.

30) From the \( E^\circ \) value, say which direction the half-reaction runs spontaneously, and one sentence justifying your answer.
31 (1.5pts) The Krebs cycle results in the production of 3 distinct energy-rich molecules. What are they?

__________________ ___________________ ___________________

32 (3pts) CO₂ is produced by the Krebs cycle, along with more useful things. Write the first reaction of the Krebs cycle that produces CO₂. Include the name of the enzyme, the names of all the reactants and products, and the structures of the pathway molecules involved.

33 (1.5pts) A later Krebs cycle reaction produces the molecule succinate. Show the reaction that produces succinate, including the enzyme name, and the names of all the reactants and products. No need for structures in this reaction.

34) Suppose you are studying the Krebs cycle in isolated cells using labeled molecules that can be detected. If 50 µmoles of acetyl groups are consumed by the Krebs cycle in one of your experiments, how much CO₂ is produced in that experiment? 

35 (2pts) What are the two enzymes of the Krebs cycle that produce CO₂ in the course of the reactions they catalyze?

First enzyme: ________________________________

Second enzyme: ______________________________

36 (3pts) List the three enzymes in the Krebs cycle that undergo allosteric regulation?

1) ________________________________

2) ________________________________

3) ________________________________

37) How many reactions of the Krebs cycle use oxygen as a substrate? ___________
The glyoxylate cycle in pathogen virulence (10 points)

In 2001 Lorenz and Fink (Nature (2001) 412 83-85) demonstrated that a pathogenic fungus needs the glyoxylate cycle to efficiently kill its mammalian hosts. Furthermore, the tuberculosis bacterium also requires the glyoxylate cycle to be fully infectious (McKinney et al., Nature (2000) 406, 735-738). These are very important observations, since processes that are needed by pathogens but absent in us are potential targets for clinical intervention. So you can see that the things we talk about in class can have medical relevance.

38) What is the glyoxylate cycle? Write one sentence about its function in the organisms that are lucky enough to have it.

The glyoxylate cycle has two unique enzymes that are distinct from Krebs cycle enzymes. They are called isocitrate lyase, and malate synthase.

39 (3pts) Write the reaction catalyzed by isocitrate lyase, including the structures of the pathway molecules.

40) From what you know about the regulation of the glyoxylate and Krebs cycles, what would you expect the activity of isocitrate dehydrogenase to be if the activity of isocitrate lysase in the same cell is high? One sentence, please.

41 (3pts) Write the reaction catalyzed by malate synthase. Include structures of the pathway molecules.

42) For each turn of the glyoxylate cycle, how many acetyl groups enter? _______________

43) For each turn of the glyoxylate cycle, how many CO₂ are produced? _______________
Gotta have glycolysis… (14 points)

44 (4pts) During glycolysis, two different phosphorylated molecules are produced that are capable of transferring phosphate to ADP to make ATP. Draw the structures of these two molecules below, in the order they are produced in the pathway.

FIRST
SECOND

45 (2pts) Write the names of the enzymes responsible for the production of each of these high energy phosphorylated molecules.

FIRST ENZYME
SECOND ENZYME

46 (6pts) When glucose is first broken into two molecules, the result is two different three carbon molecules. Draw their structures below and say the full names.

47) Each of these molecules has a phosphate, yet glucose, from which their carbons came, has not phosphate. What molecule(s) do the phosphates on each molecule come from?

48) In microorganisms, ethanol is produced when cells are primarily using glycolysis for energy. Similarly, lactic acid is produced in animal tissues that are mainly using glycolysis for energy. In both cases, the production of these molecules must occur for glycolysis to proceed. Why? (One sentence)
That other glucose pathway…. (7 points)

The pentose phosphate pathway is another fate for glucose that is distinct from The G Word, but still very important.

49 (2pts) The pathway has a first phase of 5 reactions, that involves oxidation. What are the two main, useful products of this part of the pentose phosphate pathway?

________________________  __________________

50 (3pts) The first reaction of the pentose phosphate pathway involves a very important enzyme called glucose-6-phosphate dehydrogenase. Write the first reaction, including the structures of the pathway molecules. Hint: a lactone is a cyclical ester.

51 (2pts) Which two large biomolecules, central to life, are made from one of the products of the pentose phosphate pathway?

________________________  and  __________________
One small step for a mitochondrion, one giant leap for mitochondria… (9 points)

Here are 4 cellular locations, including the three compartments of the mitochondrion

A  mitochondrial outer membrane
B  mitochondrial inner membrane
C  mitochondrial matrix
D  the cytosol

(5 pts) For each phrase below, write the one letter that is best associated with the phrase

52) ________ permeable to small ions and molecules
53) ________ location of glycolytic enzymes
54) ________ where lactic acid is formed.
55) ________ location of Krebs cycle enzymes
56) ________ location of glyoxylate cycle, if present
57) ________ location of cytochromes
58) ________ where ubiquinone is found
59) ________ contains porins
60) ________ highly impermeable to small molecules and ions
61) ________ where the PDH complex is found

62 (4 pts) Sketch a cross section of the mitochondrion below, labeling the key parts.