Lecture 1 Short problems

1) What is the one letter and the three letter abbreviations for the poly peptide shown?

2) How many peptide bonds does this polypeptide possess?

3) Circle the peptide bonds

4) Indicate the N terminus and the C terminus of this polypeptide

5) “The DNA-encoded gene for a protein directly determines its primary structure”
   Is this true, and what does it mean?

6) Can a protein have a tertiary structure but no quaternary structure? (one sentence)

7) Describe in the simplest terms possible how the R groups are arranged in an alpha helix and a beta sheet. Remember this is NOT structural biochemistry class.

8) Why do we call an enzyme a catalyst? (one sentence)

9) Does an enzyme hasten a reaction rate more in the forward direction than in the reverse direction? (Yes or no, and one sentence).

10) What is the effect of an enzyme on the “reaction coordinate” in which free energy is plotted against progress of the reaction from substrate (reactant) to product?

11) What are the four general types of catalytic mechanisms used by enzymes?
Lecture 2 Short problems

1) Along the reaction coordinate, where does one find the transition state of a reaction?

If an enzyme decreases the activation energy, $\Delta G^\ddagger$, by the quantity $L$, we learned that the forward rate constant is increased by multiplication with the factor $e^{L/RT}$.

2) Why does this simple fact show that small changes in activation energy give big changes in catalyzed rates?

3) When the activation energy is decreased by the quantity $L$, what is the effect on the reverse rate constant?

4) So is the ratio of the forward rate constant and reverse rate constant changed with and without enzyme catalysis?

5) At equilibrium, what is the fraction of binding sites occupied by a ligand in terms of the dissociation constant $K_d$, and the concentration of ligand $L$?

6) What is the value of this fraction at $L=0$? What is the value of this fraction as $L$ gets very big…?

7) What are the units of $K_d$?

8) Using this fraction, what concentration of $L$ (in terms of $K_d$) do you need to see 50% occupancy of the binding sites?

9) What concentration of $L$ (in terms of $K_d$) do you need to see 90% occupancy of the binding sites?
10) What is the analogous equation for the effect of substrate concentration (S) on the fraction of maximal rate for an enzyme that obeys Michaelis-Menton kinetics?

11) What is the value of this fraction when substrate is zero? When substrate is very high?

12) What are the units of Km?

13) What concentration of substrate (in terms of Km) is needed to see 50% saturation of enzyme rate? How about 90%?

14) Now include the other terms (Vo, Vmax) to express the full Michaelis-Menton equation, including the fraction equation from 4a.

15) Two people study the same enzyme. They get different Vmax in each experiment, but the same kcat. Why?

16) Which three of the four general mechanisms of enzyme catalysis are used in the chymotrypsin catalytic cycle?

17) How do the three catalytic triad residues (Asp, His, Ser; or D, H and S) function in the catalytic cycle of chymotrypsin?
Lecture 3 Short problems

1) What class of enzymes catalyzed the transfer of phosphate from ATP to proteins?

2) Write the general reaction for this class of enzymes. You can use an “OH” on the protein as the recipient site, and just ATP and ADP.

3) What class of enzymes catalyze the removal of phosphate from phosphorylated proteins produced by the enzymes in questions 1 and 2?

4) Write the general reaction for these “phosphate removing” enzymes. You can use as a substrate Pr-O-PO$_3$ as the phosphorylated proteins.

5) Is the dephosphorylating reaction described above the exact reverse of the phosphorylating reaction? Yes or no, and why

6) Why doesn’t the Michealis-Menton equation apply accurately when dealing with a cooperative or allosteric enzyme?

7) Draw a simple S vs rate plots for an allosteric enzyme with no additions, with an added allosteric activator, and an added allosteric inhibitor. Three curves, one graph.
8) From what you learned about chorismate mutase, draw a simple diagram showing the allosteric effects of the two amino acids tryptophan (Trp; W) and tyrosine (Tyr; Y) on the activity of this branchpoint enzyme.

9) Draw a Lineweaver Burke plot for a Michaelis-Menton enzyme. Indicate the “x” and “y” variables, the x intercept and the y intercept. Now add a second enzyme to the graph with a higher Km and a lower Vmax.

10) Memory refresher! For the reaction A + B → C + D, write the expression For the equilibrium constant
Lecture 4 Short problems

1) What is the value of $e^0$ (I mean the number “e” raised to the zeroth power)?

2) So what is the value of ln1?

3) And what is the value of $-RT\ln(1)$?

4) If $K$ is <1, what sign is $-RT\ln(K)$? What about when $K$ is >1?

5) Write the complete reaction for hydrolysis of ATP to ADP and Pi (no need for structures), and write the $\Delta G^{\circ}$ of 30.5kJ/mole next to it.

6) Write the reverse reaction, in which Pi and ADP combine to form ATP and H$_2$O. What is the $\Delta G^0$ for this reaction?

7) One of the molecules that can drive the formation of ATP is phosphoenolpyruvate (PEP). When PEP loses a phosphate it becomes pyruvate (Pyr). Write the hydrolysis reaction for PEP losing a phosphate and becoming Pyr (no structures needed) Write the $\Delta G^{\circ}$ next to the reaction, which is -61.9 kJ/mole.

8) Now, calculate the $\Delta G^0$ for PEP transferring a phosphate to ADP, to yield Pyr and ATP. Do this by writing the hydrolysis of PEP (again, just like in c), and the reaction in b) in which ATP and H$_2$O are made from ADP and phosphate. Write the correct $\Delta G^{\circ}$ next to each, paying attention to the signs. Combine these reactions into one below:

9) Calculate $\Delta G^0$ for the transfer of Pi from PEP to ADP, by using the $\Delta G^{\circ}$ for each reaction used to make the composit. Is it a spontaneous reaction?
10) Glucose-6P (G6P) has a $\Delta G^\circ$ of ~ -13.8 kJ/mole. Will the transfer of Glu-6P to ADP to make Glu and ATP be spontaneous or not? What is the $\Delta G^\circ$ of that reaction. This is exactly the same situation as 8) but with a different phosphate donor.

11) For the written reaction, which reactant is the oxidizing agent, and which is the reducing agent (in an earlier version of the problem set, I had the Co and the Co$^{2+}$ switched..sorry!)

$$\text{Co} + 2\text{Fe}^{3+} \rightarrow \text{Co}^{2+} + 2\text{Fe}^{2+}$$

12) In the reaction above, which reactant is getting oxidized, and which is getting reduced?

The picture is from the lecture slides. It is a few of the standard half-cell reactions from Table 13-6 in your book (and the previous lecture slide), and includes the extreme $E^\circ$'s, and some middle ones.

13) Which reduction is the most spontaneous; meaning which reduction is associated with the most negative $\Delta G^\circ$?

14) Which oxidation reaction is the most spontaneous?
15) Pick the two reactions that when combined as a balanced redox reaction will produce the most free energy. Write that balanced reaction

16) For your “uber reaction” (the one in 15 that gives off the most free energy), what is the $E^{\circ}$ for that combined reaction?

17) How would you convert this $E^{\circ}$ into the corresponding $\Delta G^{\circ}$
Lecture 5 Short problems

1) Using NAD+ and NADH (no structures needed) write out the reaction for the oxidation of methanol (CH3-OH) to formaldehyde, in which NAD+ is the oxidizing agent. We won’t encounter this reaction, but it is a lot like ones we will.

2) Now do the same for the oxidation of formaldehyde to formic acid (HCO2-). You can use water to balance the reaction.

3) This is strictly from The Name Game: Draw the following structures. Some of these we have encountered in our discussions. Some we have not seen, and will never see:

a) glycerol:

b) hydroxyacetone:

c) glycerol-2-phosphate:

d) glyceraldehyde-3-bromide:

e) 2-phosphoglycerate:
4) The enzyme aldolase cuts fructose 1,6 bis phosphate into a 3 carbon ketone and a 3 carbon aldehyde. This is the reverse of an “aldol condensation”, and that is why the reaction is called aldolase.

a) Suppose the 3 carbon of Fr 1,6bP is labeled (use and asterisk). Draw the labeled product from the aldolase reaction.

b) Now draw the molecule that results from triose phosphate isomerase (TPI) converting this labeled substrate into product.

5) TPI catalyzes interconversion of DHAP and G3P. Write TPI reaction with the substrate being glyceraldehyde-3-P, showing (an arrow is fine) which carbon started out as the 2 carbon in G3P.

6) You feed a cultured mammalian cell line glucose labeled at the 5 carbon. A couple minutes later, you solubilize the culture, and use a mass spectrometer to see where the label is. You find that the label is in both DHAP and G3P. Why?
7) Draw the structure of 1,3-bisphosphoglycerate (1,3bPG). Indicate which phosphorylated carbon is part of a carboxyl group and which phosphorylated carbon is part of an alcohol group.

8) Which phosphoryl group has a ΔG of hydrolysis sufficiently negative to spontaneously drive the formation of ATP from ADP?

9) Pyruvate has three carbons. Which is the most reduced, and which is the most oxidized?
Lecture 6 Short problems

1) Both lactate formation and ethanol formation have a common product. What is that common product, and why is it important?

2) Write the half cell reaction for the reduction of pyruvate to lactate, using only electrons and H+ as needed. You have never seen this in class. But it will be a learning and growth experience!

3) What are the advantages of glycolysis compared to more complete oxidation of glucose?

4) What are the disadvantages of glycolysis compared to more complete oxidation of glucose?

5) Which two reactions of glycolysis are examples of substrate level phosphorylation of ADP? You can site the enzymes, or describe the reactions, or write them out. Whut- uver…
6) Why do these two examples of substrate level phosphorylation proceed spontaneously even though phosphorylation of ADP to ATP is highly endergonic?

7) Fructose from the diet is metabolized by the liver. It is phosphorylated at the 1 position, and then cleaved by an aldolase similar to the one used in glycolysis. Draw this Fr-1P cleavage reaction, showing the structure of the substrate and the products. Include the names of the two products.

8) One of the products of the above aldolase reaction of Fr-1P is not a glycolytic intermediate. Which one?

9) Propose the simplest reaction you can think of to convert that non-glycolytic intermediate to a molecule that is part of glycolysis. (hint.. you may need to expend some energy)

10) ATP is an allosteric inhibitor of PFK, and ADP is an allosteric activator. Why does this make sense?
Lecture 7 Short problems

1) Draw a generic (using R1 and R2) ester, and show how it is formed from an alcohol and carboxylic acid. (If you haven’t learned this yet in Ochem, then the answer key will be your way of doing so.)

2) Now, draw a generic lactone. This is a cyclic ester, so just include the Cs at the alcohol and carboxylic acid groups, and use a line as the remainder of the molecule.

3) Indicate which two carbons on 6-phosphogluconate (the product of lactonase) are part of the carboxylic acid or part of the alcohol in the bond found in the substrate of lactonase.

4) What are the two main “useful” products of the oxidative pentose phosphate pathway?

5) This is a picture of ribose. Indicate which carbon number of the glucose molecule each ribose carbon comes from when synthesized by the pentose phosphate pathway. Meaning, if a ribose carbon comes from glucose carbon 3, label it “3”.

6) Now, indicate with an arrow which ribose carbon will be part of a carbonyl (aldehyde) group when the molecule is in its linear form.
7) Using just the business end of lipoic acid (the 5 membered ring with two sulfurs; I provide an example to use), draw the half reaction that converts it from the oxidized form to the reduced form. You can use H+ to balance the reaction. Just the half reaction.

\[ \text{Sulfurs} \]

8) Using the reduced form of lipoid acid (the open-ring product of the \( \frac{1}{2} \) reaction above) now depict the dehydration condensation for acetic acid with this reduced form.

9) Where in the PDH complex enzyme reactions does this condensation product you drew in question 8 above appear as a product? Where in PDH reactions does the acetyl-lipoate adduct you drew in question 8 appear as a substrate?

10) Draw the citrate molecule, and show the two acetyl groups in that molecule. Is the carbon they are attached to optically active or not?

11) Where do those two identical acetate groups come from in the newly-made citrate molecule?
12) Draw glutarate. Do it from what you learned in The Name Game (O (2), M (3), S (4), G (5) “Old Man Spittin’ Game”, or Ochem Makes Students Groan, or whatever) about dicarboxylic acids. Indicate with an arrow where the 2, or alpha, carbon is.

13) Now draw alpha-keto glutarate. Which is the same as 2-ketoglutarate, which is the same as 2-oxoglutarate, but most people will (and do) call it alpha-ketoglutarate

14) When isocitrate become alpha keto glutarate, an oxidation and a reduction occurs. Which molecule gets oxidized, and what gets reduced?

15) How are alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase similar? If you think about each as cousin alpha-ketoacid dehydrogenases, the massive similarities make sense.

16) Succinyl-CoA is the substrate for succinyl-CoA synthetase. What trinucleotide is formed in this reaction? Using just names, not structures, write the reaction for this enzyme as it is depicted in the Krebs Cycle.
17) The succinyl-CoA synthetase reaction is spontaneous ($\Delta G^\circ = -2.9$). But we are converting GDP into GTP, which is almost identically difficult as converting ADP into ATP. So, where does the free energy to drive GTP synthesis come from?

18) From the Name Game, 
(O (2), M (3), S (4), G (5) )
write the structure of succinate.

19) Now write the half reaction for succinate oxidation to fumarate. Just the half reaction, but write the structure of the product and substrate.

20) Biochemistry is full of things that sound the same by are different. Malonate is a simple dicarboxylic acid, and is part of our Name Game acronym (O, M, S, G). Malate is a four carbon dicarboxylic acid. Draw these two structures.

21) You can tell I am fanatical about the Name Game. It will pass, so bear with me. Anyway, you now know the structure of succinate. (O, M, S, G). Draw two variants of this simple molecule:

a) 2- or $\alpha$ - OH succinate:

b) 2- or $\alpha$ – keto succinate:

22) Which Krebs cycle molecules are these two structures?
Lecture 8 Short problems

1) Draw citric acid, and draw acetyl CoA (just the acetyl group; no need for the CoA, but including the S is helpful). Indicate with an arrow which two carbons could be from the CH₃ of the acetyl group added by citrate synthase.

2) Look at the picture of citrate. The circle indicates a carboxylic acid that was part of the substrate OAA from which this citrate was formed.

   a) Indicate the CO₂⁻ group that also was part of the OAA from which this citrate was formed.

   b) Indicate the carbon that was the CH₃ group of acetyl CoA

   c) Indicate the O that was the keto group on OAA

   d) finally, use a pen to trace the carbon skeleton of the OAA molecule used to form citrate by citrate synthase.

3) If you look at the anapleurotic reactions in terms of carbon number, a theme emerges. Every reaction does the same thing to carbon number of the substrates and products. What is that thing that they all have in common?

4) Let’s compare two anapleurotic enzymes. The malic enzyme converts pyruvate into L-malate. Pyruvate carboxylase converts pyruvate into OAA. Write the structures of OAA and malate (don’t worry about the L), the products of each of these enzymes.
5) One of the to anapleurotic enzymes in question 4 uses NADH or sometimes NADPH as a substrate, and the other does not. Why does this make sense from the structures of the products?

6) Suppose you are operating a single Krebs cycle. While you observe this single Krebs cycle, it turns 500 times. Answer the following questions to improve your intuition about carbon flow. It really helps to figure this out and not peek at the answer sheet. (Which I know is just a foolish professor fantasy…)

a) How many acetates were consumed by the 500 turns?

b) How many acetate carbon atoms entered the Krebs cycle during the 500 turns?

c) How many CO₂ molecules were produced during the 500 turns?

d) How many CO₂ carbon atoms were produced during the 500 turns.

e) When the 500 turns is complete, how many OAA molecules are present?

f) You overhear a student going “I don't get it, we put 500 acetyl groups into that single Krebs cycle, and at the end of it, why don’t we have 500 OAAs? What kind of lame metabolic pathway is this anyway?” Explain
Lecture 9 Short Problems

1) When a triglyceride is consumed, intestinal cells break it down into its parts and then reassemble new triglycerides. Draw a generic triglyceride, showing the fatty acids as hydrocarbon squiggles with a carboxyl group.

2) Now, show the products of complete hydrolysis of this generic triglyceride.

3) The three carbon molecule that results from the complete hydrolysis of triglycerides is called what? Draw it to the right...

4) Describe the path by which a free fatty acid is taken up by a cell and oxidized. Say the key steps that happen to this molecule and where they happen. Just words and just a few sentences.

5) What is the new (meaning new to us, rather than CoA-SH) carrier molecule involved in getting fatty acids to the site of their oxidation?

6) During the cyclical oxidation of fatty acids, called beta oxidation, four reactions occur to acyl-CoA. Describe them in words below.

7) Now show the structures that occur during fatty acid oxidation. You only need show up to the beta carbon, since the other just sit there watching in horror...

8) What set of Krebs cycle reactions is this process most like? Would you say they are chemically identical or enzymologically identical?

9) For a 20 carbon fatty acyl-CoA, how many CoA-SH are needed to fully convert this molecule to actyl-CoA?

10) In the peroxisome, the electrons that are removed in the first oxidation step of beta oxidation produce a potentially dangerous molecule. What is this molecule? Write the reaction that shows its formation?
11) What enzyme breaks down the potentially dangerous molecule produced during beta oxidation in the peroxisome?

12) Write the net reaction for synthesis of ketone body acetoacetate. Now, how many acetyl CoA actually participate as substrates in the set of reactions?

13) What is the proposed function of ketone bodies produced by the liver?

14) A boy was found to have a deficiency in mitochondrial HMG-CoA synthase (NEJM 337:1203 (1997)). Let's do a few questions about this young patient.
   a) HMG-CoA stands for 3-hydroxy-3-methyl-glutaryl-CoA. Let's figure out what this looks like, from what you already know. Remember OMSG? What is glutaric acid, or glutarate. Draw that:
   
   b) Now draw 3-hydroxy-3-methyl-glutarate (this is HMG!)
   
   c) Finally, draw 3-hydroxy-3-methyl-glutaryl-CoA
   
   d) Why can't this patient make ketone bodies even when AcCoA is abundant in the liver?
Lecture 10 Short problems

1) What are the four main parts or regions of the mitochondrion? List them:

2) Where in the cell does glycolysis occur? Where in the cell does the PDH reaction occur, and where in the cell does the Krebs cycle occur?

3) Compare the properties of the outer membrane and inner membrane of the mitochondrion. What is the key difference, if you had to choose only one feature?

4) Where is pyruvate generated during metabolism of glucose (generated means produced). Where is pyruvate oxidized into acetyl-CoA? From what you know about the mitochondrial inner membrane, what process might be required before pyruvate made in glycolysis is metabolized to AcCoA?

5) Suppose pyruvate could not get into the mitochondrial matrix. What would you expect to see occur during continued metabolism of glucose? What would have to happen for glycolysis to continue occurring in this situation?
6) Ubiquinone has a fully oxidized and a fully reduced form, called Q and QH₂. (shown in picture). Using just these terms, write the half reaction for the complete reduction Q. Just the half reaction. You can use protons and electrons as the other “substrates”.

7) Write the half reaction for the reduction of Fe³⁺ to Fe²⁺ (I had these switched on an earlier version… sorry)

8) Write the balanced reaction for NADH reducing Q. What complex catalyzes this reaction?

9) Write the balanced reaction for succinate reducing Q. What complex catalyzes this process, and what is the Krebs cycle enzyme name for this complex?

9) Write the balanced reaction for QH₂ reducing Fe³⁺ in a cytochrome c to Fe²⁺ (I had these switched on an earlier version… sorry)
10) Write the half reaction for the reduction of $O_2$ into $H_2O$. Just the half reaction, using $e^-$ and $H^+$ as needed.

11) Write the full balanced reaction for the oxidation of $Fe^{2+}$ in reduced cyt c (the free protein that has gained so much press due to its role in programmed cell death) by $O_2$, the last reaction of the electron transport chain.

12) The flow of electrons through the electron transport chain looks like 2 roads the become one, meaning it has this kind of structure $\approx$. Using the complex names I, II, III, and IV, NADH, Succinate, Q, the complexes, cytc, and O2, draw this flow of electrons. This is very important to understand.

13) There is a single “product” of the ETC that is produced by complexes I, III, and IV, that is the source of energy for ATP formation. I use quotations because it would not be traditionally thought of as a product. But we will. What is it, and why is it important?