Lecture 1 Short problems

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

YG4FV or Tyr-Gly-Gly-Pro-Leu (had Val on orig key... sorry!)

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?

Yes!
The 1^o structure is defined as the sequence of a protein which is totally determined by the DNA sequence

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

Yes, if a protein is folded (3^o) by un-associated with other folded proteins (4^o), then this is the case!

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.

In alpha helix, R groups are perpendicular to the cylinder defined by the helix. In beta sheet, the R groups point up and down perpendicular to the sheet surface.

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

Because it changes the rate of the reaction without altering the SA, or the Ka.

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

No. It hastens the forward and reverse reaction to exactly the same extent.

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?

The “hump” that represents the activation energy is lowered.

11) What are the four general types of catalytic mechanisms used by enzymes?

1) Entropy reduction 2) acid-base catalysis 3) metal ion catalysis 4) covalent intermediate
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 $e^{L/k_BT}$.

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

The exponential relationship means that small changes in activation energy cause big changes in rate. Boom!

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

The reverse rate constant is also increased by a factor of $e^{-L/k_BT}$.

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

\[
\text{Uncatalyzed ratio } = \frac{h_f}{h_r}, \quad \text{Catalyzed ratio } = \frac{h_f e^{L/k_BT}}{h_r e^{-L/k_BT}} = \frac{h_f}{h_r}
\]

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 $L$?

\[
f = \left( \frac{L}{L + K_d} \right)
\]

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

at $L=0, f=0 \quad \text{as } L\to\infty, f\to1$

7) What are the units of $K_d$?

$K_d$ is in units of concentration

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

If $L=K_d$, the $f = \frac{K_d}{K_d + K_d} = \frac{1}{2}$!

9) What concentration of $L$ (in terms of $K_d$) do you need to see 90% occupancy of the binding sites?

If $L = 9K_d$, the $f = \frac{9K_d}{9K_d + K_d} = \frac{9}{10} = 90\%$
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?

\[
f = \frac{S}{S + K_m}
\]

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

- \( f = 0 \) when \( S = 0 \)
- \( f \rightarrow 1 \) as \( S \rightarrow \infty \)

12) What are the units of \( K_m \)?

\( K_m \) is in units of concentration.

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

- If \( S = K_m \), rate is 50% of max
- If \( S = 9K_m \), rate is 90% of max

14) Now include the other terms (\( V_o \), \( V_{\text{max}} \)) to express the full Michaelis-Menton equation, including the fraction equation from 4a.

\[
V_o = \left( \frac{S}{S + K_m} \right) V_{\text{max}}
\]

(\( V_o \) is the "initial rate" right after substrate is added to the experiment)

15) Two people study the same enzyme. They get different \( V_{\text{max}} \) in each experiment, but the same \( k_{\text{cat}} \). Why?

\( V_{\text{max}} = k_{\text{cat}} E_T \), where \( k_{\text{cat}} \) is an intrinsic property of the enzyme, and \( E_T \) is the amount used in the experiment. They used different amounts of enzyme!

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

Chymotrypsin uses entropy reduction, acid-base chemistry and covalent intermediate formation.

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

- Ser - provides nucleophile \( \text{OH}^- \); His accepts and donates \( \text{H}^+ \)
- Asp - helps stabilize pos charged His
Lecture 3 Short problems

1) What class of enzymes catalyzed the transfer of phosphate from ATP to proteins?  
   \[ \text{PROTEIN KINASES transfers } P_i \]
   \[ \text{from ATP to proteins} \]

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.

   \[ \text{Prot} + \text{OH} + \text{ATP} \rightarrow \text{Prot} - O-P-O^{-} + \text{ADP} \]

3) What class of enzymes catalyze the removal of phosphate from phosphorylated proteins produced by the enzymes in questions 1 and 2?  
   Protein PHOSPHATASES catalyze the removal of \( P_i \) from phosphorylated proteins by hydrolysis

4) Write the general reaction for these “phosphate removing” enzymes. You can use as a substrate \( P-O-PO_3 \) as the phosphorylated proteins.

   \[ \text{Prot} - O-P-O^{-} + H_2O \rightarrow \text{Prot} + OH + P_i \]

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

   NO. PROTEIN PHOSPHATASES do not regenerate ATP. They catalyze the \( H_2O \)-dependent removal of phosphate.

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

   In the case of an allosteric enzyme, the catalytic behavior changes as a function of \( S \), so there is no constant \( K_m \). At each \( S \) conc., the enzyme action changes.

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:

$$K_{eq} = \frac{[C]_{eq} \times [D]_{eq}}{[A]_{eq} \times [B]_{eq}}$$
Lecture 4 Short problems

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

\[ e^0 = 1 \]

2) So what is the value of $\ln 1$?

\[ \ln 1 = 0 \]

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

\[ -RT\ln 1 = -R \times 0 = 0 \]

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

If $K < 1$, $-RT\ln(K) > 0$; when $K > 1$, $-RT\ln(K) < 0$

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

\[ \text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{PO}_4^- \quad (\text{or } \text{Pi}) \quad \Delta G^\circ = -30.5 \text{ kJ/mole} \]

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

\[ \text{ADP} + \text{PO}_4^- \rightarrow \text{ATP} + \text{H}_2\text{O} \quad \Delta G^\circ = +30.5 \text{ kJ/mole} \]

7) One of the molecules that can drive the formation of ATP is phosphoenoylpyruvate (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.

\[ \text{PEP} + \text{H}_2\text{O} \rightarrow \text{Pyr} + \text{PO}_4^- \quad \Delta G^\circ = -61.9 \text{ kJ/mole} (!!) \]

8) Now, calculate the $\Delta G^\circ$ 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:

\[ \text{PEP} + \text{H}_2\text{O} \rightarrow \text{Pyr} + \text{PO}_4^- \quad \Delta G^\circ = -61.9 \]

\[ \text{ADP} + \text{PO}_4^- \rightarrow \text{ATP} + \text{H}_2\text{O} \quad \Delta G^\circ = +30.5 \]

\[ \text{PEP} + \text{ADP} \rightarrow \text{Pyr} + \text{ATP} \quad \Delta G^\circ \rightarrow \text{see next question!} \]

9) Calculate $\Delta G^\circ$ 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?

\[ \Delta G^\circ \text{ for PEP transferring } \text{PO}_4^- \text{ to ADP to regenerate ATP: } -61.9 + 30.5 = -31.4 \]

**HIGHLY SPONTANEOUS!**
10) Glucose-6P (G6P) has a ΔG° 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 ΔG° of that reaction. This is exactly the same situation as 8) but with a different phosphate donor.

The -13.8 kJ/mole released is not large enough to run the uphill ATP formation reaction of +30.5 kJ/mole:

\[ \text{Glu-6P} + \text{ADP} \rightarrow \text{Glu} \text{ ATP} \quad \Delta G^{\circ} = +16.3 \text{kJ/mole} \]

Non Spontaneous!

11) For the written reaction, which reactant is the oxidizing agent, and which is the reducing agent:

\[ \text{Co}^{+2} + 2 \text{Fe}^{+3} \rightarrow \text{Co}^{+} + 2 \text{Fe}^{+2} \]

Co is the oxidizing agent, Fe^{+3} is the reducing agent.

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

Co is getting oxidized (losing e⁻)
Fe^{+3} is getting reduced (gaining e⁻)

13) Which reduction is the most spontaneous; meaning which reduction is associated with the most negative ΔG°

\[ \frac{1}{2} \text{O}_2 + 2 \text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2\text{O} \quad \text{E}^{\circ} \text{ is most positive} \rightarrow \text{most neg } \Delta G^{\circ} \]

14) Which oxidation reaction is the most spontaneous?

The bottom reaction, when run from right to left

\[ \text{ferredoxin} (\text{Fe}^{+2}) \rightarrow \text{ferredoxin} (\text{Fe}^{+3}) + \text{e}^- \text{ has most positive } E^{\circ} = -(-432) = +432 \]
15) Pick the two reactions that when combined as a balanced redox reaction will produce the most free energy. Write that balanced reaction

\[ \frac{1}{2} \text{O}_2 + 2 \text{Fe(II)} + 2 \text{H}^+ \rightarrow \text{H}_2\text{O} + 2 \text{Fe(III)} \]

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?

\[ E^{\circ}_{\text{full reaction}} = 0.816 + 0.432 = 1.248 \]

17) How would you convert this \( E^{\circ} \) into the corresponding \( \Delta G^{\circ} \)?

\[ \Delta G^{\circ} = -2F(1.248) \]
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.

\[
\text{CH₃-OH} + \text{NAD}^+ \rightarrow \text{H}_2\text{C} = \text{O} + \text{NAD} + \text{H}^+
\]

\[
\left( \text{CH₃-OH} + \text{NAD}^+ \rightarrow \text{H}_2\text{C} = \text{O} + \text{H}^+ + \text{NAD} \right)
\]

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

\[
\text{H}_2\text{C} = \text{O} + \text{NAD} + \text{H}_2\text{O} \rightarrow \text{H}_2\text{C} = \text{O}^- + \text{NAD} + 2\text{H}^+
\]

\[
\left( \text{H}_2\text{C} = \text{O} + \text{NAD} \rightarrow \text{H}_2\text{C} = \text{O}^- + \text{H}^+ + \text{NAD} \rightarrow \text{H}_2\text{C} = \text{O}^- + \text{NAD} + 2\text{H}^+ \right)
\]

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:

\[
\text{CH}_2-\text{CH}-\text{CH}_2
\]

b) hydroxyacetone:

\[
\text{CH}_3\text{C}=\text{O} - \text{OH}
\]

c) glycerol-2-phosphate:

\[
\text{CH}_2\text{OH} - \text{CH}_2\text{OH} - \text{PO}_3^-
\]

d) glyceraldehyde-3-bromide:

\[
\text{CH}_2\text{OH} - \text{CH} = \text{CH}_2 - \text{Br}
\]

e) 2-phosphoglycerate:

\[
\text{CH}_2\text{OH} - \text{CH} = \text{CH}_2 - \text{CO}_3^-
\]
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 an 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?

Because although the original product in glycolysis is G3P labeled at the 2 carbon, TPI will allow some of this to become DHAP, also labeled at its 2 position, by the readily reversible reaction in #5 above...
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 $\Delta G$ of hydrolysis sufficiently negative to spontaneously drive the formation of ATP from ADP?

The $\text{P}_i$ bond to the carboxyl group has a very negative $\Delta G^\circ$ (or cellular $\Delta G$ for that matter); its hydrolysis provides more than enough energy to form ATP from ADP. (The chemical reason is that this is a type of acid anhydride, very reactive.)

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?

The common product in both of these fairly distinct processes is \textit{NAD}^+, that is regenerated from NADH. Restoration of \textit{NAD}^+ is needed for continued glycolysis. No \textit{NAD}^+, no glycolysis!

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!

\[
\text{CH}_3-\text{C}-\text{CO}_2^- + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{CH}_3-\text{CH}-\text{CO}_2^- + \text{H}_2\text{O}
\]

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

\textbf{Glycolysis delivers energy more rapidly (is capable of greater power) than oxidative metabolism, and can occur in the complete absence of oxygen (O}_2).}

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

\textbf{Much less efficient use of energy stored in glucose; production of problematic byproducts (EtOH, or lactate).}

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…

\[
\text{ADP} + \text{CH}_2-\text{CH}-\text{CO}_2^- \rightarrow \text{ATP} + \text{CH}_3-\text{CH}-\text{CO}_2^- \quad \text{(1,3BPG)}
\]

\[
\text{ADP} + \text{CH}_2-\text{CH}-\text{CO}_2^- \rightarrow \text{ATP} + \text{CH}_3-\text{CH}-\text{CO}_2^- \quad \text{(P3P)}
\]
6) Why do these two examples of substrate level phosphorylation proceed spontaneously even though phosphorylation of ADP to ATP is highly endergonic?

The hydrolysis of the $\text{PO}_4$ group being transferred (which is the reaction that the donors 1,3-BPG or PEP undergo) yields far more free energy than that needed to run ATP hydrolysis backwards, that is, from ATP to ADP.

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?

Glyceraldehyde is not a glycolytic intermediate, while DHAP, the other 3 carbon product, is.

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)

The most direct route for glyceraldehyde to be included in the glycolytic pathway would be to convert it into G3P:

$$\text{CH}_2\text{-CHOH}-\text{CHOH}-\text{CH}_2^+ + \text{ATP} \rightarrow \text{CH}_2\text{-CHOH}-\text{CHOH}-\text{PO}_4^+ + \text{ADP}$$

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

When there is abundant ATP, there is no need to initiate further glycolysis. Conversely, when ATP is depleted (indicated by $\text{ATP} + \text{AMP}$), then glycolysis is needed. Now! ATP = life!
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.)

\[ R_1 \cdot CH_2-CH_2-OH + HCO_2 \cdot R_2 \rightarrow R_1 \cdot CH_2-O-C=O + H_2O \]

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?

The two commonly cited products, used in many metabolic circumstances, are: ribose-5P and NADPH.

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 from to the reduced form. You can use H+ to balance the reaction. Just the half reaction.

\[
\text{ HS } \quad + \quad 2e^- + 2H^+ \rightleftharpoons \quad \text{ HS } \quad + \quad 2H^+
\]

8) Using the reduced from 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.

\[
\text{ HS } \quad + \quad \text{ CH}_3\text{COOH} \quad \rightarrow \quad \text{ HS } \quad + \quad \text{ CH}_3\text{C}-5 \quad + \quad \text{ H}_2\text{O}
\]

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?

**Acetyl lipate is a product of the E1 reaction, where it is formed, and a substrate of the E2 enzyme, where it provides the acetyl group for transfer to CoA-SH.**

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?

One comes from OAA (it is part of the OAA structure; \( \text{CO}_2^- - \text{CO}^- - \text{CH}_2^- \text{CO}_2^- \)) and the other come from the acetyl-CoA.
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.

\[ \text{CO}_2\text{--CH}_2\text{--CH}_2\text{--CH}_2\text{--CO}_2 \quad \text{or} \quad \text{CO}_2\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CO}_2 \]

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.

\[ \text{CO}_2\text{--CH}_2\text{--CH}_2\text{--CO}_2 \quad \text{or} \quad \text{CO}_2\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CO}_2 \]

\[ \alpha \text{ is the carbon next to the functional group} \]

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

The isocitrate gets oxidized (going from an =O to an =O) and NAD\(^{+}\) gets reduced to NADH.

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.

Both lose CO\(_2\) in oxidation, and become decarboxylated to CoASH, or tricarboxylic linkage. Both use homologous E1, E2, E3 complex employing TPP, lipoate, FAD, and NAD\(^{+}\) in that order.

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.

\[ \text{Succ- CoA} + \text{GTP} + P_i \quad \rightleftharpoons \quad \text{Succinate} + \text{GTP} + \text{CoASH} \]
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? 

The hydrolysis of succinyl-CoA to succinate and CoASH is very favorable energetically.

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

\[ \text{succinate: } \text{O} - \text{C} - \text{CH}_2 - \text{CH}_2 - \text{CO}_2^- \]

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

\[ \text{succinate: } \text{CO}_2^- - \text{CH}_2 - \text{CH}_2 - \text{CO}_2^- \rightarrow 2\text{H}^+ + 2\text{e}^- + \text{CO}_2^- - \text{H}_2 \]

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.

\[ \text{malonate: } \text{C} - \text{O}_2^- - \text{C} - \text{H}_2 - \text{C}_2 - \text{CO}_2^- \]

\[ \text{malate: } \text{C}_2 - \text{O}_2^- - \text{C} - \text{H}_2 - \text{C}_2 - \text{CO}_2^- \]

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:

\[ \text{succinate: } \text{CO}_2^- - \text{CH}_2 - \text{CH}_2 - \text{CO}_2^- \]

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

\[ \text{OH} - \text{C} - \text{O}_2^- - \text{CH}_2 - \text{CH}_2 - \text{CO}_2^- \]

b) 2- or $\alpha$-keto succinate:

\[ \text{C}_2 - \text{O}_2^- - \text{C} - \text{H}_2 - \text{C}_2 - \text{CO}_2^- \]

22) Which Krebs cycle molecules are these two structures?

\[ a) = \text{MALATE} \quad b) = \text{Oxaloacetate} \]
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.

![Citric Acid and Acetyl CoA Diagram]

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?

They all use CO₂ to increase carbon number to far from a 3-carbon starting molecule.

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.

![OAA and Malic Acid Structures]
5) One of the to anaplerotic 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?

Malate is more reduced than OAA; the ketone group from pyruvate is converted (reduced) to an -OH in malate. Thus, a reducing agent is needed... Voilà NADPH

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?

500 acetates (acetyl groups) are consumed; 1 per turn

b) How many acetyl groups entered the Krebs cycle during the 500 turns?

2 carbons/acetyl x 500 = 1000 acetyl carbons

c) How many CO$_2$ molecules were produced during the 500 turns?

2 CO$_2$ made per cycle x 500 = 1000 CO$_2$ produced

d) How many CO$_2$ carbon atoms were produced during the 500 turns.

1000 CO$_2$ produced = 1000 CO$_2$ carbons

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

Only 1— we were running a single Krebs cycle... so 1

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 OAA’s? What kind of lame metabolic pathway is this anyway?” Explain

There is no net gain of Krebs cycle molecules with multiple turns. All input carbon is eventually converted into CO$_2$

7) The glyoxalate cycle (GC) is similar to the Krebs cycle, but two key reactions create a bypass that completely changes the function of this pathway.

a) The first GC reaction that differs from the KC is catalyzed by isocitrate lyase. It is a simple reaction that cleaves isocitrate into succinate and glyoxalate. Remembering the structure of succinate (OMSG), draw the structure of isocitrate and indicate with a circle the two carbons that will become glyoxalate. Also trace with a pen a line indicating the carbons that will be in the product succinate.
8) Draw the structure of glyoxalate. Just like oxalate (O M S G) is has two carbons, but it is an aldehyde. Draw it on the left in the space, because we are going to react it.

\[
\text{glyoxylate} \quad \overset{\text{CO}_2}{} + \quad \overset{\text{CH}_3}{\text{C}-\text{SGA}} \quad \overset{\text{OH}}{} \quad \overset{\text{CO}_2}{} + \quad \overset{\text{H}}{} + \quad \overset{\text{CO}_2}{\text{OASM}}
\]

9) The second unique reaction of the GC condenses acetyl-CoA with glyoxalate by the enzyme malate synthase. The chemistry here is EXACTLY analogous to citrate synthase. In both cases, the methyl group of the acetyl group attacks a carbonyl, resulting in an OH. (Check out the similarities). Then use the space above to draw the remaining parts of the malate synthase reaction, using acetyl-CoA.

10) In the full reaction in space 8 above, indicate in the product, with an arrow, what carbon was the methyl group of the acetyl CoA substrate.

11) Let's do some glyoxalate cycle bookkeeping. Remember the 500 Turns of Dr. Krebs? (sounds very Halloween). Let’s do the same thing for the CC, shown to the left. Suppose you are watching ONE glyoxalate cycle turn 500 times:

a) How many AcCoA are consumed in 500 turns of a single GC?

1000 AcCoA consumed

b) How many succinate molecules are produced in the 500 turns?

500 Succinate made

c) How many carbon atoms enter as acetyl groups during the 500 turns?

\[2 \times (1000 = 2000 \text{ acetyl carbons} \text{ enter})\]

d) Write the net reaction for the 500 turns of the GC discussed above:

\[1000 \text{ AcCoA} \quad 500 \times GC \quad 500 \text{ Succinate} + 1000 \text{ OASM}\]

e) How many CO2 are produced during the 500 turns of the GC?

No CO2 is produced! NADA!

f) Finally, how many OAA are present after the 500 turns of one GC?

One OAA is present after 500 GC turns.
Lecture 9 Short problems

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

- Outer membrane
- Intermembrane space
- Inner membrane
- Matrix

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?

Glycolysis occurs in the cytosol while the PDH reaction that generates AcCoA occurs in the mitochondrial matrix.

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.

The outer membrane is quite porous, with a MW cutoff of ~5000 daltons (big). In contrast, the inner membrane is quite impermeant.

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?

Since the pyruvate is produced in the cytosol, it must get across the inner membrane to be processed by PDH. There must be a transporter protein that allows pyr to cross the IM.

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?

There would be a buildup of pyruvate in the cytosol, and AcCoA production in the matrix would not occur, so no Krebs cycle. In order for glycolysis to continue, NAD+ has to be regenerated by converting pyr to lactate.
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”.

\[ Q + 2e^- + 2H^+ \rightarrow QH_2 \]

(But Q can go through two separate reductions)

\[ Q + e^- + H^+ \rightarrow QH^- + e^- + H^+ \rightarrow QH_2 \]

7) Write the half reaction for the reduction of Fe³⁺ to Fe²⁺.

\[ Fe^{3+} + e^- \rightarrow Fe^{2+} \]

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

\[ \text{NADH} + H^+ + Q \rightarrow NAD^+ + QH_2 \]

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?

\[ \text{Succinate} + Q \rightarrow \text{Fumarate} + QH_2 \]

Note: In the Krebs cycle, we say that COOH₂ is the product, and in complex II, QH₂ is the first acceptor in complex II, but then the e⁻, which goes to cytochrome c to Fe²⁺, is said to go to cytochrome c.

9) Write the balanced reaction for QH₂ reducing Fe³⁺ in cytochrome c to Fe²⁺.

\[ QH_2 + 2Fe^{3+} \rightarrow Q + 2Fe^{2+} \]
10) Write the half reaction for the reduction of \( O_2 \) into \( H_2O \). Just the half reaction, using \( e^- \) and \( H^+ \) as needed.

\[
\frac{1}{2}O_2 + 2e^- + 2H^+ \rightarrow H_2O \\
(\text{or } O_2 + 4e^- + 4H^+ \rightarrow 2H_2O)
\]

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.

\[
2 \text{Cyt}^{\text{red}} + \frac{1}{2}O_2 + 2H^+ \rightarrow 2 \text{Cyt}^{\text{ox}} + H_2O \\
(\text{or }) \\
2 \text{Fe}^{2+} \text{cyt}_c + \frac{1}{2}O_2 + 2H^+ \rightarrow 2 \text{Fe}^{3+} + H_2O
\]

12) The flow of electrons through the electron transport chain looks like 2 roads the become one, meaning it has this kind of structure >—<. Using the complex names I, II, III, and IV, NADH, Succinate, Q, the complexes, and \( O_2 \), draw this flow of electrons. This is very important to understand.

\[
\text{NADH (I) } \rightarrow \text{Q (II) } \rightarrow \text{Cyt}^{\text{red}} (III) \rightarrow \text{Cyt}^{\text{ox}} (IV) \rightarrow O_2, H_2O
\]

(Note—both I and II use Q as a substrate. They operate independently, and each contributes to the pool of QH\(_2\).)

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?

Each of the complexes, in the course of moving electrons, actively transports \( H^+ \) from the matrix side of the inner membrane, to the IMS side of the inner membrane, creating an \( H^+ \) gradient and actual electrical potential. THIS GRADIENT PROVIDES THE POWER TO DRIVE ATP SYNTHESIS.

(Sometimes this substrate-product relationship is expressed as \( H^+ \) for \( H^+ \) on the “negative side” and \( H^+ \) for those moved to the “positive side.”)
Lecture 10 Short problems

1) Draw the mitochondrial inner membrane (just a bilayer) with indicators of the IMS and the matrix sides. Now indicate the proton gradient, and the direction that protons are transported during the action of the ETC.

2) On your map above, Show the direction that protons move when they are used to make ATP. Just direction please.

3) When a weak organic acid is added to mitochondria, what is the effect on the proton gradient? Why does a weak organic acid have this effect?

4) In the “in vitro” isolated mitochondria coupling experiments we discussed in class (and in your book), the first electron source is usually succinate, due to the experimental convenience of using this reagent. Draw a cartoon of the ETC in the inner membrane as it operates when succinate is added to active mitochondria. Include the pumping of H+ to form the proton gradient. Indicate the two sides of the inner membrane.
5) When NADH is used as a proton source, the resulting proton gradient is bigger than when succinate is used. Why is this so? Redraw the little cartoon in 6 to indicate the flow of electrons and the proton pumping that results from NADH being used as an electron source.

![Cartoon of electron and proton flow](image)

6) What are the two components of the equation for the free energy stored in the proton gradient called? And what do they physically refer to? What is the significance of each term being a separate entity in the equation?

\[ \Delta G = 2.3RT \Delta pH + 2F \Delta \psi \]

The \( \Delta pH \) term indicates the concentration gradient which will provide energy, and the \( \Delta \psi \) term is the voltage difference, which also provides energy. The fact that they are separate terms means they can independently contribute to ATP synthesis.

7) If glycolysis is running forward, you can measure it by looking at the disappearance of glucose, or the appearance of pyruvate. For each situation, describe what happens to glucose disappearance and pyruvate production, both from glycolysis

a) NAD+ is removed: glucose disappearance stops, pyruvate appearance stops

b) ADP is removed: glucose disappearance stops, pyruvate appearance stops

c) free PO4 is removed: glucose disappearance stops, pyruvate appearance stops
8) Now answer the same question for ADP disappearance and ATP production for the following situations during glycolysis:

a) NAD+ is removed:
   - ADP disappearance stops
   - ATP production stops

b) Glucose is removed
   - ADP disappearance stops
   - ATP production stops

c) free PO₄ is removed
   - ADP disappearance stops
   - ATP production stops

9) Suppose you are observing glycolysis in a yeast cytosol preparation, just like Buchner. It is running along making all the expected products, consuming glucose. Suddenly, you add an inhibitor of pyruvate kinase, and you add enough to completely inhibit that enzyme.

a) You notice that the reaction 2PG → PEP + H₂O? rapidly stops, even though the inhibitor is for the next enzyme downstream. Why does this happen?
Since the consumption of PEP is blocked (PEP is substrate of the inhibited enzyme), PEP builds up until the reaction no longer goes forward... ΔG → 0 for PEP generating Rxn.

b) Similarly, you notice that every reaction has stopped. Why?
Eventually, all of the products build up and each reaction stops. ANALOGIES: 1) An accident on the highway will back up traffic for miles. 2) A bend in a hose will stop water flow at every point upstream. 3) Someone conversing with the ticket agent at the theatre will stop everyone from getting a ticket.

c) Now, to the inhibited mix, you add an enzyme that converts PEP into oxaloacetate, and everything needed to make that happen. Soon you observe all the upstream glycolytic steps working again. Why?
Lessening PEP levels by this new alternative will now allow other upstream reactions to flow.

a) Glu →... → PEP → PPy → Pys pathway flows
b) Glu →... → PEP × PEP blocked, whole pathway stops
   PEP lowered by alternate deliprotein, upstream rxns resume
   c) Glu →... → PEP × OAA
10) In a distinct experiment, you are observing isolated mitochondria oxidizing succinate, reducing $O_2$, and making ATP. You have ADP, Pi, and succinate present, and as expected, while succinate is being converted into fumarate, $H_2O$ is being formed, and ADP is being converted to ATP.

a) You add an inhibitor of ATP synthase called oligomycin. ALL of these processes stop. Why does blocking ATP production stop succinate from being oxidized? Don’t say, “’cause they’re coupled”. What is the molecular cause of the upstream inhibition?

b) To this inhibited mitochondrial mix, with the oligomycin still present, you add a weak organic acid that carries protons across membranes, from regions of higher concentration to lower concentration. Now what happens to succinate consumption, $H_2O$ production, and ATP production?

c) Draw a little cartoon of the three cases: uninhibited, + oligomycin, and +oligomycin + proton carrying drug. Show the proton gradient in each, using the number of $H^+$ you draw to indicate gradient magnitude.

d) This problem is EXACTLY analogous to problem 9. And the proton carrying drug in this problem (that relieves the block to ETC) is exactly analogous to the PEP-consuming enzyme used in 9c to alleviate the block. Why do I say this?