Metabolic Biochemistry Midterm
Thursday November 6, 2014; 8pm...

One function of this exam is to solidify your knowledge of the commonly used core pathways and ideas found in metabolism. The other is to estimate how much you have learned so far. We can best serve these two distinct purposes by making the scoring the midterm so that it can only help you: if averaging in the midterm helps you at the end of the quarter (meaning if it is a higher percentage score than the final), we will use it in calculating the scoring. If it hurts you (meaning you got a better percent grade on the final than the midterm)… it is not used! So the course score it is more about what you have learned rather than exactly when you learned it.

We are not about tricking you or trying to be crafty and fooly. This is a very straightforward exam, and what we ask for is what we want. Period. Enjoy, to the extent that this is possible in a midterm...

Our only advice: Please please PLEASE read the whole question before you start your answer. And don’t hesitate to ask questions. That is why we are circulating around like glycolytic Coelacanths who must answer questions to survive...

Summation

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Total _________(out of 100)
1 (5pts) My plate is full… Hey look! Like I advised, it’s a license plate for practice of the amino acid one letter code. First, match up the letters on the license plate to the correct full amino acid names. For those names that are not represented on the plate, put “none”.

a) Cysteine c
b) Proline p
c) Alanine none
d) Phenylalanine F
e) Arginine R
f) Histidine H
g) Serine S
h) Glutamate E
i) Pargiline none

j) Which AAs on the license plate have a net positive charge at normal cell pH? __________
k) Which two AAs on the license plate are part of the chymotrypsin catalytic triad? ________
l) Which AAs on the license plate have an aromatic ring in the R group? _____
m) What is the function of each of the two amino acids you mentioned in k) in the catalytic triad? (one sentence)

2 (9pts) … Enzoom!
Two students are studying the same enzyme. In fact, they have each been given a sample of the enzyme from the same bottle. They both perform experiments measuring the initial velocity (Vo) as a function of substrate for their enzyme, and discover this is a simple Michaelis-Menton enzyme. Thus, they each get values for Km, Vmax, kcat. When they are writing their lab reports, they find that their two Km values are the same, and their two kcat values are the same, but the Vmax for the two experiments differs by a factor of two. Answer the following questions:

a) How could the Vmax values be different yet the kcat values be the same in these studies? (one sentence)

b) Draw a Lineweaver-Burke plot using the provided axes, showing the results of two experiments, indicating the two axes, and where the Km and Vmax values are for each experiment.

c) Both students agree that at 3µM substrate, the enzyme is functioning at ¼ maximum velocity. What is the numerical value of the Km from this information? Hint: remember that f= S/(S+Km)

\[
\frac{3}{(3+Km)} = \frac{1}{4} \quad \text{by inspection} \\
\text{This is a Michaelis-Menten enzyme, so expect non-Sigmoidal Rectangular hyperbola, with constant } K_m \text{ on constant } V_{max}
\]
3 (7pts) **Enzymes are proteins of action:** In class we have examined the reaction energy diagram many times, which shows the free energy for a substrate becoming a product.

a) Using S and P as the substrate and product, draw a reaction energy diagram for the exergonic (spontaneous) reaction S to P, and indicate the effect of an enzyme that catalyzes this reaction on the energy graph. Indicate S, P, the $\Delta G$ for the reaction, the $\Delta G^\ddagger$ for the reaction, and any changes to these quantities caused by the enzyme. Use the space provided to the right....

a) Suppose and enzyme causes a $10^{15}$ fold rate enhancement to the S to P reaction. What would you predict the enhancement to the reverse reaction to be and why (One sentence)

4 (9pts) **A hex upon you!** In a fascinating report, Christesen et al. (DIABETES, VOL. 51, APRIL 2002) describe a human mutation of the liver form of hexokinase (called glucokinase). It is a single mutation in which V456 is replaced by A, that changes the activity of the enzyme. Lets learn about this mutation by first thinking about the enzyme.

a) Complete the hexokinase reaction, given the structure of the main product. Include other substrates and products. Include the names of the substrate(s) and product(s).

b) What metabolic pathway is this reaction part of? **Glycolysis**

Here is what the Christesen paper says about the A456V mutant form of liver hexokinase: "A456V result[s] in lowering $S_{0.5}$, lowering the Hill coefficient for the substrate..., and increasing the $k_{cat}$. The activation results in a near-hyperbolic, high-$k_{cat}$, and low $S_{0.5}$ enzyme that contrasts with the catalytically less active sigmoidal wild-type." Fancy. Just to clarify, "$S_{0.5}$" is the substrate concentration that results in 50% maximal activity. “Near-hyperbolic” means the shape of the curve is like our old friend, the Michaelis-Menton enzyme.

c) Here is a graph from the actual paper comparing the mutant enzyme to the normal enzyme: the $k_{cat}$ is plotted at various substrate concentrations. From the description above, which is the mutant enzyme curve, A or B? Why do you say this (one sentence)

d) Patients with the mutant enzyme have hypoglycemia, that is, abnormally low levels of blood glucose. Why does that make sense? One sentence.
5 (6pts) Breaking Sweet  Glycolysis literally means “breaking glucose”. There is a step in the pathway where a six carbon molecule is broken into two smaller molecules, catalyzed by the enzyme aldolase.

a) Complete the aldolase reaction given the structure of the six carbon molecule. Include the names of the substrate and the products and the structures of the products.

b) Where did the two phosphates on the pictured substrate come from? 

(c) The next enzyme after aldolase is abbreviated TPI. What does TPI stand for? 

d) Which aldolase product is oxidized by NAD+ in the next glycolytic reaction?

6 (6pts) Lifting prohibition... Many organisms convert pyruvate into ethanol. This is done in two steps. First pyruvate is converted into acetaldehyde by pyruvate decarboxylase. Then the product of that reaction is converted into ethanol by alcohol dehydrogenase. Let’s think about this process using the Socratic method.

a) Complete the two reactions below, given the structure of acetaldehyde, including the structures of the listed molecules, and the names of unlisted substrates and products. Use the curved arrows included for graphical convenience. Sorry about the scrunchy look...

b) Where is the TPP cofactor involved in this sequence of reactions, and what does it do? (one sentence)

c) This reaction sequence has an important role in allowing continued glycolysis. What is that role? (one sentence)

d) Mammals like us accomplish the same function with a distinct reaction of pyruvate catalyzed by lactate dehydrogenase. Write that reaction to the right, with the same level of detail as the set above.
7 (5pts) The other fate of pyruvate... When pyruvate is going to be oxidized by mitochondria, it undergoes conversion by the pyruvate dehydrogenase complex (PDH).

a) What is the PDH product that is used to feed 2 carbon units into the Krebs cycle? _______________

b) TPP, FAD, and lipoic acid are three key cofactors in the action of the PDH. Pick TWO and describe their roles in the PDH catalytic cycle (one sentence each):

1): Lipoic acid -sulfurs accept electrons and acyl group from pyruvate to form thioester intermediate

2): TPP - provides a nucleophile to attack pyruvate carbonyl

FAD - reoxidizes reduced lipoic acid back to disulfide form

c) Pyruvate has three carbons; two of them end up in a product bound for the Krebs cycle. What is the fate of the third carbon of pyruvate (one sentence)? Draw pyruvate in the box, and show us that carbon.

The carboxyl carbon is lost as CO₂


d) Along with the product in a, what is the other significant product of the PDH reaction?

NADH (some also added CO₂)

Take a breath….then proceed with the exam. Space below reserved for serene thoughts
8 (12.5pts) Parts and labor (this is a greatest hit) Often glycolytic intermediates are simply mentioned as abbreviations. The box on the left is a "parts list" of molecules in glycolysis.

a) Using these abbreviations, and arrows for each enzyme-catalyzed step, draw a reasonable depiction of glycolysis. I provided glucose. Use all the parts. It helps to remember which things have six carbons and which have three. Make sure to include NAD, ATP, Pi, or ADP where needed, using curved arrows to reduce clutter. Include "Lac" even though it is technically after glycolysis. Remember there is a branched reaction...

b) Using the abbreviations in the list, and any other reactants or products needed, write the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase, one of my favorite enzymes. No structures needed:

\[
G_3P + \text{NAD}^+ + \text{Pi} \rightarrow 1,3\text{bpg} + \text{NADH}
\]

9 (3pts) What's my name!... For the following dicarboxylic acid names (you have seen this before I suspect) write the correct number of carbons.

a) Glutaric 5 Oxalic 2 Malonic 3 Succinic 4

b) Now, we are going to draw a structure, using the name game. You will learn that the molecule \(\alpha\)-hydroxy-gluutarate is very important in some cancers. Draw the structure of this molecule in the space to the right. You have never seen this molecule, yet you know its structure!

10 (4pts) When glucose goes rogue! The pentose phosphate pathway (PPP) is another important route of glucose usage in the cell. It is particularly important in anabolism.

a) What is anabolism? (one sentence):

Anabolism—the set of metabolic pathways that construct larger molecules from smaller ones.

b) What are the two main products of the PPP? \(\text{NADPH}\) and ribose-5p, ribulose-5p, ribose, etc.

c) The following lactone is produced in the first step of the PPP by the indicated enzyme. Complete the reaction providing the structure of the starting 6 carbon metabolite, and names of other important products or substrates:

\[
\text{G6P dehydrogenase} \rightarrow \text{6-phosphogluconolactone} + \text{NADPH} + \text{H}^+\]

d) What is the eventual fate of the 1-carbon, marked with an arrow, in the PPP? (one sentence):

It is released as \(\text{CO}_2\) (thus resulting in the ribose carbon skeleton).
10 (8.5 pts) Hans Krebs would rather direct.... This looks familiar. Like I said, there are only so many ways to ask about these classic reactions. For each question answer with the molecule number that is the best answer. You can use a single number, more than one, or put “none” if none apply:

a) Which metabolite is produced along with GTP? 6
b) Which metabolite undergoes CO$_2$ loss during the Krebs cycle? 3,4
c) Which metabolite is produced by an enzyme that is similar to aKG Dehydrogenase? 1

d) Which metabolite is created by reducing FAD? 7

e) Which 4 carbon metabolite undergoes oxidation by NAD$^+$? 8

f) Which metabolite has 6 carbons and 2 –CO$_2$ groups? 4, 9

g) Which metabolite is an alpha-keto acid? 4, 9

h) Which metabolite is a symmetrical molecule? 2, 6, 7

i) Which metabolite is produced by aKG Dehydrogenase? 1

j) Which metabolite has a thioester bond? 1, 5

11 (4pts) A Popular Pattern In the Krebs cycle, a pattern of 3 reactions is observed all over the place in metabolism, including the Krebs cycle. The pattern goes: 1) oxidation to form a double bond, 2) addition of water to that double bond to form an alcohol, 3) oxidation of the alcohol. Draw the three Krebs reactions, in order, that show this common pattern. Include the structures and names of the metabolites. Include the names of reactants and products distinct from the Krebs cycle metabolites. You don’t need to name the enzymes. I will even give you the starting product...(the saturated carbons are simply angles in this representation)

12 (6pts) The Gly-whatylate Cycle?! It seems like just yesterday that we spoke about the glyoxylate cycle, which is a variant of the Krebs cycle used to make larger carbon molecules from smaller ones. Let’s contrast and compare the GC to the KC.

a) How many acetyl groups enter the Krebs cycle per turn? 4

b) How many acetyl groups enter the glyoxylate cycle per turn? 2

c) How many CO$_2$ exit the Krebs cycle per turn? 2

d) How many CO$_2$ exit the glyoxylate cycle per turn? 0

e) What four carbon molecule is produced by each turn of the glycoxylate cycle? succinate

f) Is the molecule in e) also made during the Krebs cycle? Yes (but it is then consumed)

g) Write the net reaction of the glyoxylate cycle below: no structures needed, just names:

h) So why is is that acetate can not be used for building bigger carbon molecules with the Krebs cycle but can be used for this purpose using the glyoxylate cycle? (One sentence)
13 (5pts) Sketchy mitos make us hyper We have started our mitochondrial odyssey with lecture 9. To understand the energetics of this incredible organelle, it is important to understand the structure.

a) Draw a picture of a mitochondrion showing the inner and outer membrane, the ancient DNA that tells us it comes from an uninvited guest, the locations of the PDH, the Krebs cycle, and glycolysis and the ETC

14 (4pts) It's a lineup! For each structure, write its name and the pathway where it arises in the spaced provided.

<table>
<thead>
<tr>
<th>Name</th>
<th>Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>fumarate</td>
<td>Krebs</td>
</tr>
<tr>
<td>G3P, glycolaldehyde</td>
<td>3-P</td>
</tr>
<tr>
<td>αKG, alpha-keto glutarate</td>
<td>Krebs</td>
</tr>
<tr>
<td>PEP, phosphoenol-pyruvate</td>
<td>glycolysis</td>
</tr>
<tr>
<td>glycolysis</td>
<td></td>
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</tbody>
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15 (6pts) Its m’truth...(T/F)
Write T or F for each question

a) _____ If a reaction’s activation energy drops by L, the rate is multiplied by $e^{L/RT}$

b) _____ Ingested fructose is eventually converted into molecules that can be metabolized by glycolysis

c) _____ The large energy drop reactions are typically the regulated reactions in metabolism

d) _____ The Krebs cycle is used both for catabolism and anabolism

e) _____ The Krebs cycle does not employ oxygen as a substrate

f) _____ No oxidation reactions occur in glycolysis

g) _____ Mammals must regulate entry of AcCoA into either the glyoxylate cycle or the Krebs cycle

h) _____ Plants can convert AcCoA into carbohydrates with the glyoxylate cycle

i) _____ Phosphofructokinase is a regulated step in glycolysis

j) _____ Citrate is an optically active molecule with an R and an S form

k) _____ Malonic acid has more carbons than oxalic acid

l) _____ Malonic acid has more carboxyl groups than oxalic acid

m) _____ Randy can sound like, but does not look like, Olaf the Snowman...