Good evening. This is the midterm and like we discussed, it has two purposes. The first is to solidify your knowledge of the basic and oft-used concepts and information covered in this test. The second is to discern how much you know.

I have tried to make the test more about recognizing things than simply pulling them out of memory. There are a few cases where pulling out of memory is asked, but not many.

My only advice is to read the whole question before you dive in, and to feel free to ask questions. That is why we are circulating around like sharks who must move to breath. Finally, please believe me when I say we are not about tricking you, fooling anyone, or trying to be all crafty-like. 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…

Summation

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1-4 (6 pts) To the right is the classic free energy reaction coordinate, showing the S to P reaction in two situations. In one an enzyme is present, and in the other, there is no enzyme.
1 Which curve (1 or 2) best represents the reaction with the active enzyme, and why? (one sentence):

The curve 2 represents the plus enzyme curve, because the activation energy, $\Delta G^\ddagger$ is lowered.

2 An excellent if somewhat aggressive student comes to Dr. R’s door and says “This problem is incorrect, because the curve with the enzyme should have P with a lower free energy than without the enzyme!” Let’s think about that, and answer the following questions:
If the free energy of P alone were lowered, would the S to P rate be changed? (one sentence)

That would not change the S to P rate, and would increase the P to S rate!

3 If the free energy of P alone were lowered, what would happen to the Keq?

The Keq would change to a larger quantity

4 In general, what is the effect of an enzyme on the forward (S to P) and reverse (P to S) reactions?

Enzymes cause the same multiplicative change for both forward and reverse reaction. Each changes by a factor $e^{d/RT}$ where $d$ is the drop in activation energy.

5-8 (4 pts) The Ghost of Allostery The strangely familiar picture shows a “generic” linear metabolic pathway, in which A goes to F by 5 enzyme steps. The graph is an enzyme rate plot like we have discussed in class. The rate of the C to D reaction is shown, when C is alone in the reaction (“c alone”), and when two different allosteric regulators are present (curve 1 and curve 2).

5 Which curve shows the effect of an allosteric activator __2__

6 Which curve shows the effect of an allosteric inhibitor __1__

7 High concentrations of A stimulate the pathway by altering Ecd. Which curve describes A’s action? __2__

8 High concentrations of F turn down the pathway by altering Ecd. Which curve would best describe F’s action? __1__
9-11 (9 pts) This just in! Last week, the FDA indicated that the company Vertex has promising results in the fight against hepatitis C virus (currently afflicting something like 1 in 35 people on Earth). Vertex developed an inhibitor, named telaprevir, of the enzyme NS3-A4. NS3-A4 is a serine protease, similar to chymotrypsin. Here is a description of NS3-A4 from the scientific literature: “The HCV NS3-4A serine protease retains some highly conserved features of the chymotrypsin family, such as spatial location of the catalytic triad of His^{57}, Asp^{81}, and Ser^{139}, as well as the positions of backbone amides of Gly^{137} and Ser^{139}, which forms the oxyanion hole.” See... what you are learning actually has some use! Answer the following questions:

9 (3pts) For the three catalytic triad amino acids His, Asp and Ser, write the one letter code, and ONE SENTENCE describing what that amino acid does in the action of chymotrypsin:

HIS  _H_ : His transfers protons from the serine OH or from the attacking H_2O to allow acid base catalysis

SER  _S__ : Serine OH performs the actual nucleophilic attack on the peptide bond, and acquires the covalent adduct of the remaining peptide chain

ASP  _D__ : Aspartate provides charge neutralization to the His that gains a positive charge when it is transferring protons during the catalytic cycle

10 (2 pts) At Vertex, you and an assistant are each measuring the activity of NS3-A4 using a substrate S. You each are using the exact same batch of the NS3-A4. You perform a rate analysis using a 1uM concentration of the enzyme, but your assistant performs their analysis using 2uM enzyme concentration. Which measured quantities below will be the same and which will be different in your two analyses. In each space, write S for same, or D for different. That is all. No numbers.

Km ___ S ___ Vmax ___ D _____ kcat ___ S ___ Half maximal [S] ___ S ___

11 (4 pts) This is a Lineweaver-Burke plot of the results form your and your assistant’s experiment on NS3-A4. Which is your curve (A or B), and which is the assistant's curve (A or B)

Your curve: __ A __ Assistant’s curve: __ B __

Write the algebraic term for the X intercept of a Lineweaver-Burke plot:

X intercept = -1/Km
12-19 (15 pts) Parts and Labor  Most of the time that any of you encounter glycolysis in your “real lives” as physicians, scientists, biomedical professionals, people-who-respire, or wherever, you will hear or see mention of individual reactions or metabolites, and have to put them into some contextual context. With that spirit in mind, we will work this advanced version of the Coat Hanger model of glycolysis. The spaces represent the main pathway metabolites, and the numbers represent the enzyme catalyzed reactions.

12 (7 pts) Using the parts list in the box, put each listed metabolite in the correct space. All parts should be used, and there are no “wild-cards”. I already put in glucose for you, at the top.

13) Which reaction oxidizes an aldehyde ___ 6__

14) Which reaction (s) consume ATP 1, 3

15) Which reaction (s) produce ATP __7, 10__

16) Which reaction regenerates NAD+____11____

17) Which reaction is catalyzed by aldolase __4___

18) Which reaction moves a phosphate from one carbon to another ___ 8____

19 (4 pts) Choose ONE reaction, from 2 to 10 (so not 1 and not 11). Draw the complete structures of the pathway substrate and product. Include the name of the enzyme, include any cofactors (name only), and make sure the reaction is balance.

See glucose, glycolysis and krebs, or your text.
20- 30 (18 pts) Krebs Schmebs!! To the right is a depiction of the Krebs cycle, with blanks for each carbon metabolite. A parts list of each of these metabolites is provided. Each number and line segment is an enzyme and a reaction.

20 (5 pts) Fill in each blank, using the conveniently provided parts list. There are no false parts, and there are no tricks.

(21-30 comprises 13 pts)
21 What are the three cofactors that are employed by enzyme #2
   _ TPP _ lipid acid _ FAD_

22 Enzyme # 6 of the Krebs cycle is most like enz # 2?

23 Enzyme #2 is called ___Pyruvate dehydrogenase___

24 Which reaction(s) yield NADH: (do not include #1)
   ______4, 5 and 10______

25 Which reaction(s) yield CO₂ as a product? ______5 and 6___________

26 Enzyme # 8 is also called Complex II; it is named__succinate dehydrogenase__

27 Which reaction requires an H₂O molecule? # _9_

28 Which reaction produces a molecule of GTP? # _7_

29 Which enzyme is fumarase? # _9_

30 The picture to the right is one of the molecules of the Krebs cycle. What is it?
   ___Citrate, citric acid______
31-34 (10 pts) **Fruc off!** One of the most newsworthy sugars is fructose. It is found in almost all of the foods we eat. It is an isomer of glucose.

31 (3 pts) **Given the linear structure of glucose,** draw the structure of fructose right next to it.

One of the ways that fructose enters the glycolysis pathway is that it get phosphorylated on the 1 carbon by, and then cleaved by an enzyme called aldolase B. The products of the aldolase B reaction are DHAP, and glyceraldehyde. The structure of glyceraldehyde is shown to the right.

32 (2 pts) Which three carbons of fructose 1-P do you think this glyceraldehyde comes from in the aldolase B reaction? (hint... it is just like the more familiar aldolase of glycolysis)

Carbons 4, 5 and 6 will produce the glyceraldehyde while the “top” 1,2 and 3 will produce the DHAP just like in glycolytic aldolase.

33 (2 pts) What glycolytic intermediate is glyceraldehyde MOST similar to? **Glyceraldehyde-3-P** or **G3P**

34 (3 pts) Write a one step reaction that will convert glyceraldehyde into a real glycolytic intermediate. You are welcome to use any substrate (like ATP for instance) that you like

35-36 (6pts) **Two halves make a whole** Here are two half reactions from a key reaction in the Krebs cycle:

\[
\begin{align*}
\text{Fumarate} + 2\text{H}^+ + 2e^- & \rightarrow \text{succinate} & E' &= 0.031 \\
\text{FAD} + 2\text{H}^+ + 2e^- & \rightarrow \text{FADH}_2 & E' &= -0.219
\end{align*}
\]

35 (4 pts) Write the balanced redox reaction in the direction that is spontaneous.

36 (2 pts) In the reaction you wrote above, which is the reducing agent, and which is the oxidizing agent?

Reducing agent ___ **FADH**₂  Oxidizing agent ___ **Fumarate**
37- 40 (6 pts) A seedy cycle... A variant of the Krebs cycle is used to covert AcCoA into larger molecules. That cycle is called the glyoxalate cycle. It depends on two enzymes, isocitrate lysase and malate synthase. Answer the following questions about this anabolic powerhouse.

37 What is a real-life example where the glyoxalate cycle is critical? (one sentence)
When seeds germinate, much of the glucose they produce comes from glyoxalate cycle.

38 Why can the glyoxalate cycle be used to produce larger molecules like glucose from AcCoA while the Krebs cycle can not? (one sentence)
For every acetate (2C) put into the Krebs cycle, two CO2 are released.

39 Every time the glyoxalate cycle turns, two AcCoA molecules are consumed, and one four carbon molecule (see? bigger!) is generated. What is that four carbon molecule? (one word)
Succinate.

40 (3 pts) Like I said above the two key reactions of the glyoxalate cycle are isocitrate dehydrogenase and malate synthase. CHOOSE ONE and write out the entire reaction, include the structures of the main products and substrates (things like CoA can be called CoA, of course). Include the name of the enzyme so we know which one you are referring to. To help you, here is the structure of glyoxalate, which you can imagine might be part of one or both reactions...

41-43 (6 pts) Reducing with glucose
41 The pentose phosphate pathway is important for producing two key molecules for use in a variety of circumstances. What are these two major products of the pentose phosphate pathway.

_____ NADPH ____ and _____ Ribose-5-P (or just ribose) _____

42 One of the products of the pentose phosphate pathway is a lactone, derived from glucose, shown to the right. In the space provided, draw that lactone.

43 The next step of the pathway is catalyzed by lactonase (dever!). The product of this reaction is called gluconic acid. Draw that structure below. Don’t worry about stereochemistry.
Sex, Power and Suicide....It’s complicated 43-53 (15 pts)
43 (2 pts) The mitochondrion has four main structural features.
The outer membrane, the intermembrane space, the inner membrane and the matrix. Draw a simple picture of the mitochondrion showing these features

44 Where does conversion of pyruvate to AcCoA occur? ___MATRIX_____

45 Where does the production of NADH by the Krebs cycle occur? ____ MATRIX ______

46 Is pyruvate produced from glucose in the mitochondrion (yes or no) ? ___ NO (cytosol) _____

47 Where are the enzyme complexes of the ETC located? ____ INNER MEMBRANE_____

Here is a picture of a major mitochondrial cofactor:

48 What is the name of this cofactor? Coenzyme Q or Q

49 Where is it located? _ on INNER MEMBRANE_____

50 Is this the oxidized or the reduced form? ____ OXIDIZED from (vs QH2)__

51 Which complexes use this form as a substrate ____Complexes I and II_____

52 Which complex produces this form as a product? ____ Complex III_____

53 (3 pts) ATP synthase is the final enzyme of oxidative phosphorylation. It is able to catalyze the generation of ATP from ADP and Pi. What is the energy source directly used by ATP synthase to run this ATP-producing reaction spontaneously, and how is this energy source employed. (One or two sentences) ATP synthase uses the proton gradient produced by the enzymes of the ETC to power the production of ATP from ADP. The enzyme allows protons in the innermembrane space (Hp⁺) to move through the enzyme in a way that provides energy for ATP production.
54 (5 pts) Rogue’s Gallery! For each structure, write its name and the pathway where it arises in the spaced provided.

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<thead>
<tr>
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<td>ribose-5-P</td>
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<tr>
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<tr>
<td>succinyl-CoA</td>
<td>Krebs cycle</td>
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<tr>
<td>OAA, oxaloacetate</td>
<td>Krebs cycle</td>
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<tr>
<td>DHAP, dihydroxyacetone</td>
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