

Do we need a calculator for the final?

Yes.

What is the breakdown in % on the final of old material and new? Will the format be the same as our previous tests? (should I bring the same scantron?)

The final is ~50% cumulative and ~50% non-cumulative - same format. Yes, bring the same Scantron sheet (882-E).

I am confused about the PFK-2 enzyme. On slide 18 it shows the enzymatic activity (affinity for F6P) of PFK-2 is reduced when no F26BP is present. Why? If PFK-2 converts F6P to F26BP, shouldn't its activity increase when there is no F26BP in the environment?

We need to separate the functions of the two PFK enzymes:

PFK-1 is the glycolytic enzyme that we primarily want to regulate.

PFK-2 is the enzyme that produces fructose-2,6-bisphosphate, an activator of PFK-1

In the presence of F-2,6-BP, PFK-1 is more active, as shown in that figure. Varying the concentration of F-6-P and measuring the rate, is the method we use to generate a standard Michaelis-Menten plot for an enzyme. Notice the activity of PFK-1 is higher in the presence of F-2,6-BP at any substrate concentration.

Of course, you are right, F-6-P is also a substrate for PFK-2, but that is not what this figure shows, and there is no PFK-2 in this experiment – only its product, F-2,6-BP.

Can you also go over the part concerning the "single polypeptide with opposing activities (PFK-2)" and how phosphorylation of the enzyme works.

The activity of PFK-2 is reciprocally regulated with the enzyme that has its opposite activity – removing the phosphate on carbon 1 of fructose – and these two enzymes are on the same polypeptide. This is common because with a single modification, you can turn one enzyme on while turning the other off and vice versa.

Hi professor, can you give me some suggestions on how to best prepare for the upcoming midterm? Do I need to know how to draw every structure? And for the pathways do I need to draw and name every enzyme and structure for example glycolysis or TCA. For example being able to draw the complete TCA cycle or the entry of galactose into glycolysis? Any tips on what to study so I can be best prepared for the 1st midterm will be much appreciated thank you.

Okay, first you should know all structures and pathways - this is very important. Once you know the pathways, then you can really start to understand the pathways. I want you to learn how metabolism works and how molecules are interconverted to meet the needs of the organism. Try to visualize each pathway as a small part of a larger machine and how everything works together.

The enzyme that converts glyceraldehyde into glyceraldehyde-3-Phosphate is called Glyceraldehyde Kinase in Voet & Voet but its called Triose Kinase in the notes. Is there any difference?

Nope, they are the same thing. Enzyme names vary from textbook to text book based on the author(s) preferences. Some enzymes have many aliases.

How important is the fructose and galactose entry into glycolysis?

On a scale of 1 to 10 - about an 8.7

Why would fructose/galactose enter or be utilized in glycolysis?

We eat them, so they need to be metabolized. It is much better, if possible, to convert a metabolite into an intermediate in an existing pathway, rather than have a whole new pathway designed to specifically metabolize, say galactose.

Do we need to memorize the delta G values of the hydrolysis of ATP or any constants, like the gas constant R?

No, these will be given if necessary.

I had a question about fructose and galactose entry into glycolysis. I don't know when and how I would draw the entry of fructose and galactose into glycolysis.

You should prepare for questions like: "draw all of the glycolytic reactions that are necessary to convert one molecule of fructose to two molecules of pyruvate.", I recommend drawing all of the reactions that we learned from glycolysis, fermentation, and the TCA cycle connected on a large sheet of paper to really visualize how it's all connected.

I had a question about what we are required to know about PDC. Should we memorize the mechanisms that pyruvate undergoes in the PDC? What should we focus on in terms of the PDC for the final?

You do NOT have to know the reaction mechanism for the pyruvate dehydrogenase complex. Know the structures of thiamine pyrophosphate, the lipoamide sidechain, acetyl-CoA and where on each the "business" happens. Also know the order of the steps in the mechanism.

Hi Pete, I just want clarifications for glycolysis steps. Which ones are reversible and which ones are irreversible.

All are reversible (near equilibrium) except those catalyzed by hexokinase, phosphofructokinase and pyruvate kinase.

Complex II doesnt pump any protons, right?

Correct. Its job is to get the electrons into the chain.

As far as the reactions go, there are so many. How would you give us a question on the test about them could you give us a few examples.

Yes, there are a lot. You should practice drawing them whenever you get some free time. Many short periods of studying probably are better than one long memorization session. Just carry a pencil and paper around with you and draw structures and pathways when you get a few minutes. An example question might be something like:

“Draw the reactions that convert fructose 6-phosphate to phosphoenolpyruvate. Include the structures of all intermediates, what enzymes are involved and any cofactors if present.”

How much detail do you want us to know for each of the complexes?

The same level of detail as we covered in class and the notes. The You do not need to know the mechanism of reduction of O_2 by cytochrome c oxidase (but you should know the stoichiometry of the reaction).

In Glycolysis, you wrote that TPI, favors DHAP but in the next reaction you have G3P as the reactant, why would it favor DHAP if you need G3P to go on with glycolysis?

The position of the equilibrium is what it is, and depends on the thermodynamics (no enzyme can change that). This particular equilibrium happens to lie in favor of DHAP. It doesn't matter, however, because as you remove G3P, no matter how small the pool of it is, the equilibrium of $DHAP \leftrightarrow G3P$ will adjust to make up for the removal of G3P. Remember – as always - enzymes don't affect equilibrium, just the speed at which it is reached.

In the NADH-Q reductase complex, I am not sure exactly how the electrons are passed between the two types of iron-sulfer clusters on their way to reducing ubiquinone. Could you please explain?

Just think of them as a wire that conducts electricity. They pass electrons by alternating their oxidation state from Fe^{2+}/Fe^{3+} .

What is the breakdown of ATP generated from glucose?

Here's a breakdown for one glucose going through glycolysis and the TCA cycle using the **glycerol phosphate shuttle** (brain and skeletal muscle). Of course, if we use the **malate/aspartate shuttle** we will pump an additional 8 protons per glucose. This will yield another 2.5 or so ATP's.

Glycolysis:

2 ATP
2 NADH (equivalent to 2 FADH₂ - consequence of being in the cytosol)

Two pyruvate dehydrogenase reactions (PDC):

2 NADH (matrix)

Two rounds of the TCA Cycle:

2 ATP (derived from GTP)
6 NADH + 2 FADH₂ (matrix)

Each matrix NADH can lead to 8 protons being pumped out of the matrix and each FADH₂ (or cytosolic NADH) can lead to 4 protons being pumped.

8 NADH: $8 \times 8 = 64$ protons
4 FADH₂: $4 \times 4 = 16$ protons
80 total protons pumped

$$\frac{80 \text{ protons}}{3 \text{ protons per ATP}} \approx 27 \text{ ATP's}$$

(you can also say **each NADH yields about 2.5 ATP's and each FADH₂ yields about 1.5**)

If we add this value to the four ATP's we produced directly we get ~31 ATP's per glucose.

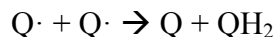
Note: this estimate varies quite a bit from textbook to textbook, but is usually in the range of 30-36 ATP's per glucose. Most textbooks nowadays show complex III pumping 4 protons instead of 2, but the total ATP's produced remains about the same, because the extra protons are offset by the ATP synthase reaction requiring ~3.3 protons per ATP instead of 3. So, it is about the same either way – *just be aware it is different in different books*. The problem lies in the fact that the stoichiometry is very hard to measure precisely. Additionally, the proton gradient is not used exclusively for generating ATP – other transporters take advantage of the proton gradient as an energy source.

In complex 3, is the purpose of cytochrome b simply to help regenerate QH₂?

Essentially, yes, but....

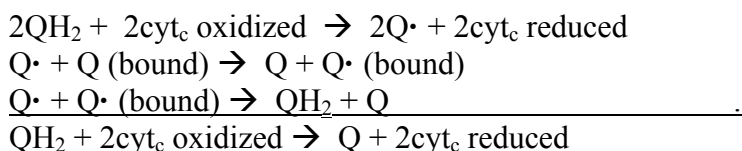
This is not a regeneration of QH₂, rather it is the *disproportionation* of 2 Q•

A disproportionation is a reaction that looks like this:



Notice this generates one Q and one QH₂, and removes two radicals (Q•).

Let's look at the stoichiometry for two electrons going through complex III:

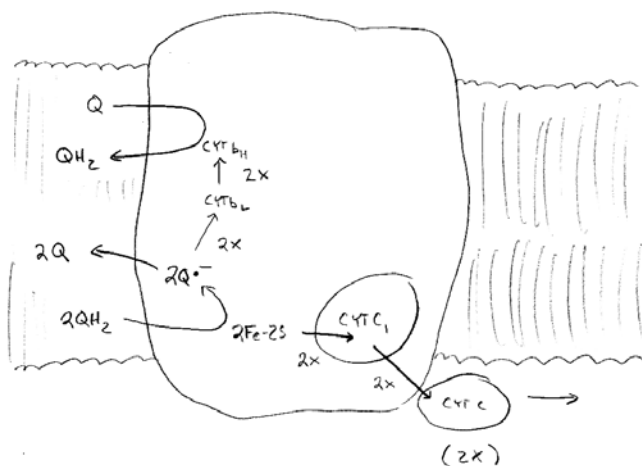


Notice:

- all Q• cancel out
- 1Q cancels out
- 1 QH₂ cancels out

This is how we get the overall equation: QH₂ + 2cyt_c oxidized → Q + 2cyt_c reduced.

I drew the following figure to (hopefully) help illustrate the paths of all the electrons in complex 3:



Every '2x' means that a single electron passes twice (remember 2 cytochrome c proteins are required to carry 2 electrons). The Q reduction occurring on the upper part of the figure represents the 'bound' Q. Try and make sense of the above stoichiometry using this figure.