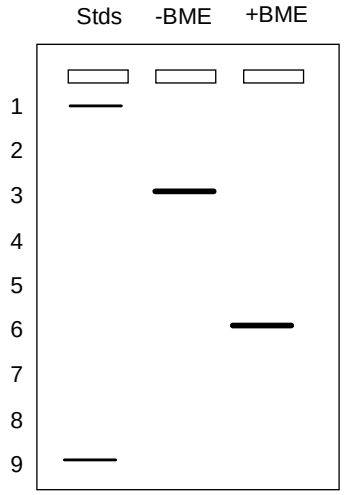
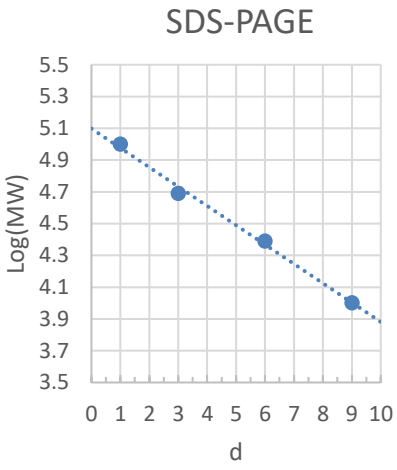
Instructions: This exam has 15 questions on 6 pages, for a total of 100 points. On questions with choices, all of your attempts will be graded and you will be awarded the highest grade. Please use the space provided or the back of the preceding page**.**

1. (12 pts) You are studying an enzyme whose predicted molecular weight (based on the amino acid sequence) is 25 kDa. You hypothesize that it may be a homodimer, with the subunits joined by a disulfide bond, e.g. α-α.

You run an SDS page gel without and with BME (shown on the right). Your standards are 10kDa and 100kDa. Does this gel ***fully*** support your hypothesis? Why, or why not?

(log 25000=4.4, log 50000=4.7)





The SDS gel + BME shows one band at 25 kDa, so it contains one or more of a single subunit – this is consistent with a homodimer (5 pts)

The SDS gel without BME shows a band of 50 kDa, so that is consistent with two chains linked by a disulfide (5 pts)

However, the quaternary structure could be $(\alpha\text{-}\alpha)_N$ so it would be necessary to measure the native molecular weight with size exclusion to fully justify the hypothesis (2 pts).

2. (6 pts) Please do **one** of the following choices:

Choice A: How does X-ray diffraction allow the determination of the atomic structure of molecules?

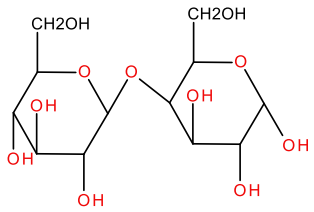
Choice B: How can a Ramachandran plot be used to determine whether a structure is correct or not?

Choice A: Interference between the X-rays scattered from the electrons on the atom depend on the relative position. The interference will affect the intensity of the scattered x-rays, therefore the intensity can be used to determine the position of the atoms (phase information is also required).

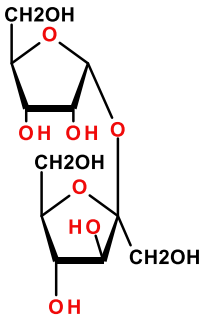
Choice B: Most of the points on the Ramachandran plot should fall in the low energy regions, indicating that the model is consistent with the expected geometry.

3. (8 pts) Please do **<u>one</u>** of the following choices:

Choice A: Draw the following sugar. β-glucopyranosyl (1-4) α-galactopyranose (galactose is an epimer of glucose at carbon 4).



Choice B: Name the sugar shown on the right.



α-ribofuranosyl (1-2) β frucofuranoside
(both anomeric are joined, therefore ends in "oside")

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4. (6 pts) Complete the following table, comparing cellulose, glycogen, and bacterial cell walls.

	Monosaccharide(s)	Linkage	Biological function
Cellulose	glucose	β(1-4)	Structural
Glycogen	glucose	α(1-4) and α(1-6) branches	Glucose storage
Bacterial Cell walls	NAM & NAG	β(1-4) & protein crosslinks	Structural

5. (6 pts) Please do **one** of the following choices

Choice A: What structures do fatty acids and phospholipids spontaneously form in water and what "force" drives this formation?

Choice B: How is a triglyceride similar to a phospholipid? How are they different? (a sketch of the chemical structure of each is an acceptable answer).

Choice A: Fatty acids form micelles (because they are cone shaped) while phospholipids form bilayers (because they are cylindrical). This is driven by the hydrophobic effect.

Choice B: Both contain the glycerol, a 3 carbon poly-alcohol. Triglycerides have a fatty acid esterified at each carbon, phospholipids have a phosphate group at carbon one instead (may have other groups attached to the phosphate.

6. (8 pts) Please do **one** of the following choices:

Choice A: Explain why corn oil is a liquid at room temperature, but margarine that is made from corn oil is a solid at that temperature.

Choice B: What effect does cholesterol have on the melting properties of lipid bilayers and why is this important biologically?

Choice A: Corn oil is a triglyceride that contains cis unsaturated double bonds, these disrupt the van der Waals interactions between the chains lowering the melting temperature. Margarine is made by adding hydrogen to the double bonds to form saturated fatty acids. These pack together well and have a higher melting temperature due to enhanced van der Waals interactions between the fatty acid tails.

Choice B: Cholesterol disrupts the van der Waals interaction between the fatty acid tails, reducing the cooperativity of the melting. This keeps membranes fluid over a wider temperature range, allowing things to diffuse in the membrane, i.e. permitting CoQ to move between complexes in the electron transport chain.

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7. (8 pts) Please do **one** of the following choices.

Choice A: Both alanine and valine are non-polar, however a protein that contains only alanine will not spontaneously insert into a membrane, but a protein that contains valine will. Why?

Choice B: Briefly describe why the K^+ channel will allow K^+ ions through a membrane, but not sodium.

Choice A: Inserting the main chain atoms into the bilayer is unfavorable because they are polar. The Alanine sidechain does not provide enough energy to overcome this, so the net standard energy for Alanine is >0, or unfavorable. The valine sidechain releases more energy (due to the hydrophobic effect), so its net standard energy is <0, indicating that insertion is favored.

Choice B: The selectivity filter, which is composed of C=O groups is just the right size to interact strongly with K^+. This interaction makes desolvation of the K ion favorable. Smaller ions interact less favorably with the C=O groups, thus the net energy change (desolvation + interaction with C=O) is unfavorable.

8. (4 pts) Select **one** of the two enzymes and briefly describe the reaction that it catalyzes. Indicate your choice.

Choice A: Kinase

Adds a phosphate group to a -OH using ATP as the phosphate donor.

Choice B: Phosphatase

Removes a phosphate group by hydrolysis, releasing inorganic phosphate (no ATP/ADP involved)

9. (2 pts) Indicate the cellular location of the following compounds or pathways or events using the following key:

C=cytosol, MM=mitochondrial Matrix, IM = Mitochondrial inner membrane

Triglycerides ___C_____

AcylCoA (fatty acid activation) ___C____

AcetylCoA ___MM_____

Electron transport ___IM_____

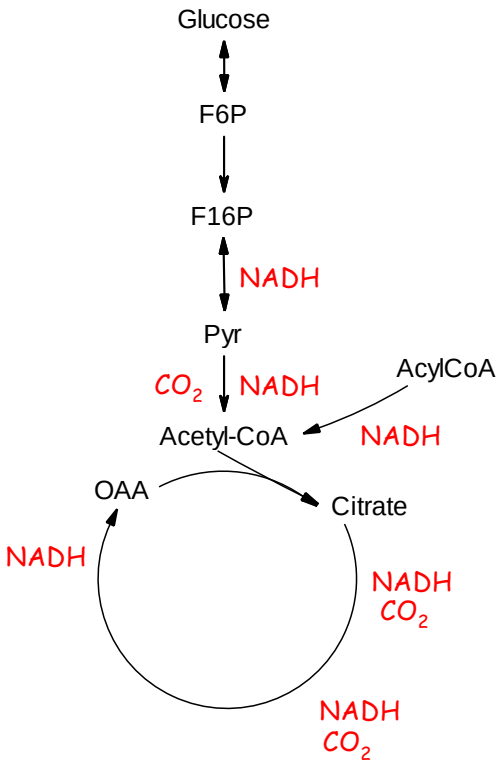
10. (8 pts) Pathway Energetics (do all parts):

i) Indicate the locations where the following are generated on the diagram to the right (2 pts)

a) NADH (6 locations)

b) CO_2. (3 locations)

ii) ***Briefly*** explain how the energy in NADH is converted to ATP (6 pts)



NADH gives its electrons to complex I in electron transport, flow of electrons is:

I→Q→III→Cytochrome C→IV→O_2

This process pumps 10 protons across the inner membrane, generating a proton gradient. ATP synthase uses the energy in that proton gradient to make ATP.

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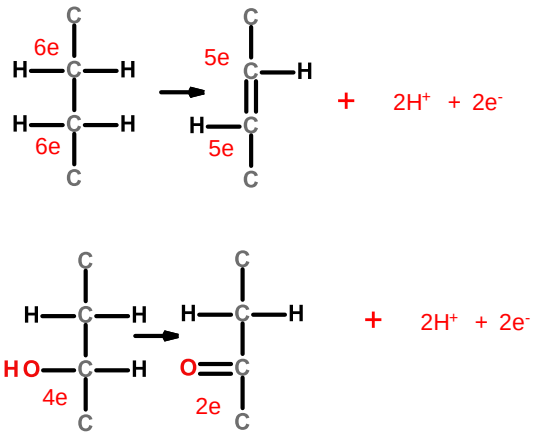
11. (8 pts) Please do **one** of the following choices:

Choice A: Redox chemistry

i) Select any **one** of the reactions shown on the right and determine whether it is an oxidation or a reduction. *Justify your answer, either by electron counting or balancing.*

The balanced equations, as well as electron counting are shown on the right. Both give the same result, 2 electrons are removed, therefore a 2 electron oxidation.



ii) What is the general name of the enzyme that catalyzes this reaction?

dehydrogenase

Choice B: Anaerobic metabolism

i) What compound is required by glycolysis that is regenerated by electron transport complex I when O_2 is available?

ii) How does the cell regenerate this compound when oxygen is absent? You can discuss either mammals or yeast.

i) NAD+ is required as an electron acceptor.

ii) Pyruvate is converted to either lactate (mammals) or acetylaldehyde and then to ethanol (yeast). In both cases pyruvate is being used as the electron acceptor instead of oxygen, converting NADH back to NAD+

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12. (6 pts) Please do **one** of the following choices. Please indicate your choice.

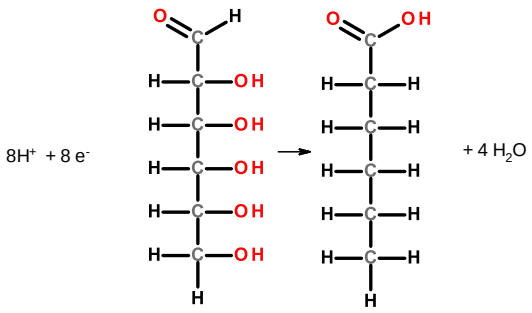
Choice A: Why are fats higher in energy content than sugars, i.e. hexanoic acid can generate more ATP than a six carbon aldose. You can answer this question by: i) a discussion of the pathways that fatty acids and glucose are oxidized, or ii) discussing the oxidation state of these two compounds, relative to CO_2.

Choice B: Describe how the amino acids alanine, aspartic acid and glutamic acid are oxidized to CO_2.

Choice A:

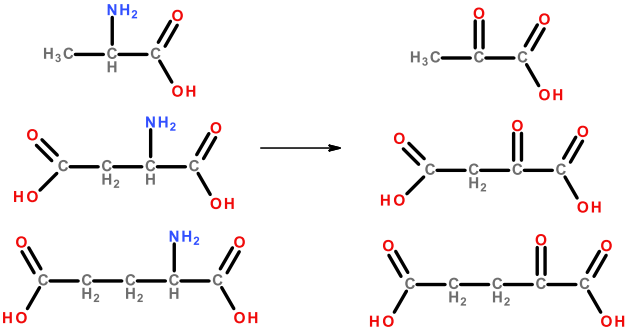
Pathway answer: Oxidation of the sugar will only produce 2 acetyl-CoA while oxidation of the fatty acid will produce 3. Although some NADH and ATP are produced in glycolysis these are smaller amounts than from an acetyl coA, so less ATP will be made.

Redox answer: To balance the above equation, you need to add 4 H2O to the right, This requires adding 8 H to the left, so to go from a sugar to the fatty acid is a reduction, since oxidations release energy, a reduction stores energy, so a fatty acid has a higher energy content.



Choice B:

The amino group is replaced by a keto group (C=O) by a transaminase. This converts alanine to pyruvate, aspartic acid to oxaloacetate, and glutamic acid to ketoglutarate, all of which are part of the TCA cycle (or the entry point to the TCA cycle, in the case of pyruvate).



13. (2 pts) Please do **one** of the following:

Choice A: When calculating the Gibbs free energy for transport, ΔG^o is assumed to be zero, why?

Choice B: What does the term ZFΔV represent in the formula for the Gibbs free energy?

Choice A: Because the same compound is on both sides of the membrane. ΔG^o is the difference in energy between products and reactants, which are the same.

Choice B: The energy associated with moved a charged ion through a voltage difference.

14. (6 pts) Select **either** direct **or** indict coupling and briefly discuss how it is used to make a pathway spontaneous. Regardless of your choice you should discuss the relationship between coupling, Gibbs free energy, and the spontaneous direction of the reaction. The following equation may be helpful:

$$\Delta G = \Delta G^o + RT \ln[B]/[A]$$

Direct: Use energy of ATP to make the standard energy negative which will make the Gibbs energy negative, making the reaction spontaneous in the forward direction. This occurs on a single enzyme and the phosphate group is often directly transferred to the other substrate.

Indirect: A favorable reaction at the end of the pathway keeps the concentration of [B] below its equilibrium concentration, which makes the 2nd term in the above equation negative, making the Gibbs energy negative.

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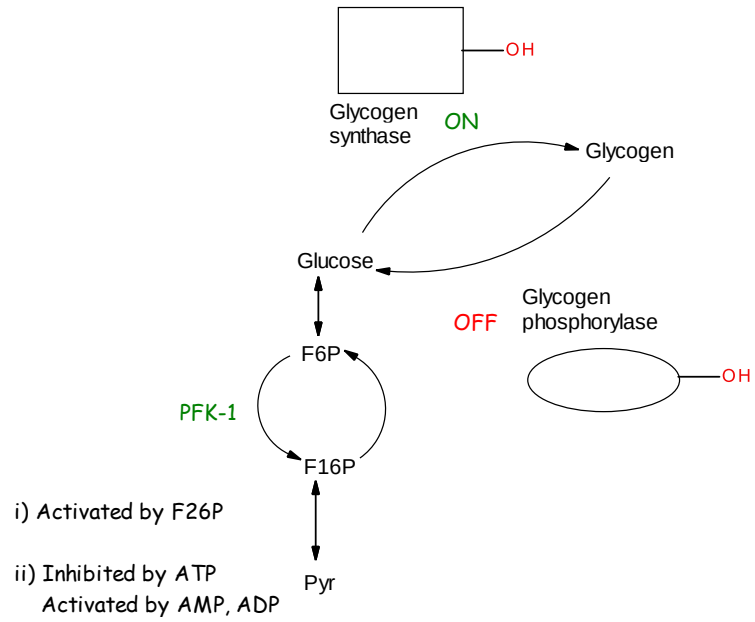
15. (8 pts) At carnival you ate way too much cotton candy (e.g. glucose) than you should have. Do both parts i and ii.

i) Briefly describe the steps in hormonal regulation of glycogen metabolism, e.g. which hormone is released, are proteins phosphorylated or not, which enzymes are is active? The diagram may be helpful to illustrate your answer (4 pts)

ii) Now do **one** of the following two choices:

Choice A: Under these conditions the liver cell may undergo glycolysis. Briefly discuss how F26P regulates glycolysis to allow this to happen (4 pt).



Choice B: Under what conditions will the liver cell actually undergo glycolysis? You should discuss how the key enzyme is regulated by adenosine compounds (ATP, ADP, AMP). Which of these compounds (ATP, ADP, AMP) is acting as a feedback inhibitor of glycolysis? (4 pt)

i)

1. The high level of glucose in the blood cause the production of insulin
2. Insulin binding to its receptor will activate phosphatases, removing phosphate groups from enzymes.
3. Glycogen synthase is ON when dephosphorylated, storing glucose in glycogen.
4. Glycogen phosphorylase (which releases glucose from glycogen) is OFF with dephosphorylated.

ii)

Choice A:

- Dephosphorylation of enzymes activates PFK-2, so levels of F26P rise.
- F26P is a required allosteric activator of PFK-1 in glycolysis, this would allow glycolysis to be on.

Choice B:

- ATP should inhibit PFK-1 because this is produced by glycolysis
- AMP and ADP should activate PFK-1 because high levels of these compounds indicate that the cell needs to replenish ATP by oxidizing glucose.
- ATP is acting as a feedback inhibitor.

Bonus 1: Coaches often advise runners to "carbo load" to provide energy during the race. Why?

This allows the runner to generate large glycogen stores in their liver to provide glucose during the race.

Bonus 2: Marathon runners, even if they do "carbo load", deplete the stored energy from the carbs ½ way through the race, what do they use as an energy source to complete the race?

Fatty acids are used, they are a rich source of energy and the energy is released fast enough if the pace of the race is relatively slow, as in a marathon.

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