This exam consists of 100 points on five pages. Note: the last question is worth 16 points.

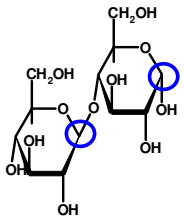
1. (4 pts) What is an "electron density map" and what is its role in structure determination by X-ray diffraction?

- An electron density map shows the location of electrons in 3D space (1 ½)
- It is obtained from the X-rays scattered from the compound (½)
- Atoms are placed into regions of high electron density, giving the 3D coordinates of the compound that originally scattered the X-rays (2 pts)

2. (5 pts) What is the difference between an aldose and a ketose? Provide **one** example.

An aldose has an aldehyde group at carbon 1 (+2 pts) – e.g. glucose, ribose, glyceraldehyde
A ketose has a ketone group at carbon 2 (+2 pt) e.g. fructose, dihydroxyacetone.
+1pt for correct example.

3. (5 pts) The structure of a disaccharide is shown on the right;



i) identify both anomeric carbons by circling them (1 pt)

ii) Which is the correct name for this compound? Briefly justify your answer.

β-glucopyranosyl (1-4) α-glucopyranose (3 1/2 pts)
α-glucopyranosyl (1-4) β-glucopyranose
β-glucopyranosyl (1-4) α-glucopyranoside (2 ½ pts)
β-glucofuranosyl (1-4) β-glucopyranose
β-glucopyranosyl (6-3) α-glucopyranose

Justification (1/2 pt)
The anomeric of the left glucose is β (-OH is pointing up)
Linkage is between carbon 1 on the left glucose and carbon 4 on the glucose at the reducing end.
Right glucose, at the reducing end, is α (-OH is pointing down)

4. (6 pts) Please do **one** of the following choices:

Choice A: Is it possible to obtain the disaccharide shown in the above question (#3) from the hydrolysis of glycogen (or starch)? Why or why not?

No – linkages in glycogen are α(1-4) the above shows a β(1-4) linkage. (6 pts)

Choice B: Briefly compare and contrast cellulose to the protein-carbohydrate (peptidoglycan) component of bacterial cell walls.

Both contain monosaccharides linked by β(1-4) (1 ½)
Both are linear chains. (1/2)
Cellulose is made of glucose - peptidoglycan modified glucose (NAG and NAM) (2 pts)
Peptidoglycan crosslinked by polypeptide chains, between NAM units. (2 pts)

5. (9 pts) Please do **one** of the following two choices:

Choice A: Which thermodynamic "force" or interaction is responsible for the self assembly of micelles and lipid bilayers? How does this interaction affect the critical micelle concentration (CMC) of fatty acids? Your answer should include a description/drawing of the structure of a fatty acid or a phospholipid.

Fatty-acid: Long chain carboxylic acid.

Phospholipid: two long chain carboxylic acids linked to glycerol, polar head group also linked.

(2 pts for structural description)

The hydrophobic effect – The non-polar acylchain releases ordered water molecules when self-assembly occurs. (5 pts)

The CMC is the highest concentration of free fatty acids, the more non-polar (longer chain) the lower the CMC since the fatty acid would enter the micelle more readily (2 pts).

Choice B: What thermodynamic "force" or interaction is responsible for the physical properties (liquid or solid, fluid or gel) of triglycerides or membranes? Illustrate your answer with an example.

These changes are due to van der Waals interactions between the acyl chains (5 pts).

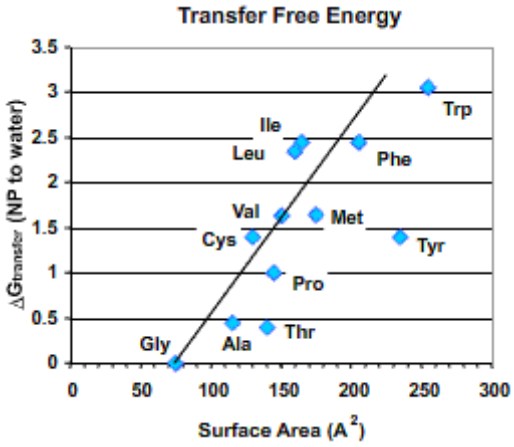
Examples (4 pts):

Increasing the acyl chain length will increase the melting temperature due to increased number of atoms that are able to participate.

Adding cis double bonds (or removing them) will lower (or raise) the melting temperature because the acyl chains will be bent or kinked by the bond – reducing van der Waals interactions.

6. (9 pts) Please do **one** of the following choices:

Choice A: Why is it difficult for ions such as Na^+ and K^+ to cross a pure phospholipid membrane? Briefly describe the mechanism by which ion channels move these ions across a membrane.



They are charged and will not be stabilized in the non-polar interior of the bilayer (4 ½ pts)

The channel provides a series of C=O groups that interact with the ion as it goes through the channel (4 ½ pts)

Choice B: Why must the secondary structure of all integral membrane proteins be either α-helix or β-barrel?

The hydrogen bond between the peptide (mainchain) and the water are lost when the peptide enters the membrane. This must be reformed to be energetically favorable, and the only suitable donor/acceptors are from the other mainchain atoms. Both an α-helix and a β-barrel fully satisfy all H-bonds. (9 pts)

Choice C: Briefly explain why a peptide composed of all alanine residues will not partition into membranes while a peptide composed of phenylalanine residues partitions almost entirely into membranes. The diagram on the right may be useful.

Inserting the polar mainchain atoms into the bilayer is unfavorable (4 pts). Although the insertion of an alanine sidechain is favorable, it isn't enough to overcome the mainchain contribution, so the overall free energy is positive. Phe has a larger non-polar sidechain and the energy released when it inserts is sufficient to overcome the polar mainchain atoms (5 pts)

7. (10 pts) Compare and contrast direct and indirect coupling and briefly discuss how these mechanisms are used to insure that a metabolic pathway is spontaneous in the forward direction. Provide **one** example.

- Both are used to change the Gibbs free energy of a reaction from positive to negative to make that step in the pathway spontaneous (3 pts)
- Direct coupling uses the energy released when ATP is converted to ADP + Pi to drive the reaction. The transfer of energy occurs within the active site of the enzyme, i.e. both ATP and the substrate are bound on the same enzyme (3 pts)
  Examples (+1 pt): Any kinase: hexose kinase, PFK-1, PFK-2.
- Indirect coupling used energy from a subsequent step in the pathway. This subsequent step has a large neg. ΔG so the concentration of all intermediates above the favorable step are kept below their equilibrium values, which can cause ΔG to become negative (3 pts)
  Examples (+1 pt): Most of the reactions in glycolysis from F16P to PEP are indirectly coupled to the large energy that is released from PEP to Pyr.
  The conversion of malate to oxaloacetate in the TCA cycle is indirectly coupled to citrate formation.
  The activation of fatty acids is indirectly coupled to the hydrolysis of pyrophosphate.
  The reactions in gluconeogenesis are indirectly coupled to the hydrolysis of F1,6 P and G-6-P

8. (6 pts)

i) Which metabolic pathway can produce ATP (energy) in the absence of oxygen? (circle correct answer):

threonine synthesis gluconeogenesis TCA cycle glycolysis fatty acid oxidation

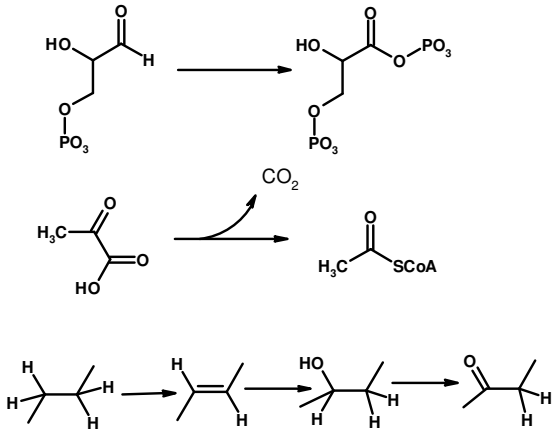
ii) What is the role of lactate (lactic acid) or ethanol in this process?

Pyruvate is reduced to these compounds in humans (lactate) or yeast (ethanol).
This allows NADH to be recycled back to NAD+ so that another cycle of glycolysis can occur.
(+4 pts)

9. (8 pts)

i) Using *any* oxidation reaction from *any* pathway, illustrate how the energy that is released by the oxidation is captured instead of being lost as heat [The diagram on the right may be helpful.] (6 pts).

ii) Which is the generic name of an enzyme that typically catalyzes reactions of this type (2 pts)?



i) For the top two reactions, the electrons released by the oxidation are placed on NADH
The first reaction of the lower series generates FADH2 and the last reaction of that series generates NADH.
(+2 pts)

NADH and FADH2 are high energy compounds that can be used to synthesize ATP.
(+4 pts)

ii) dehydrogenases catalyze redox reactions (+2 pts)

10. (5 pts) Please do **one** of the following choices:

Choice A: Compare and contrast a kinase to a phosphatase. Give **one** example.

A kinase is an enzyme that adds a phosphate group using ATP, e.g. PFK

A phosphatase removes a phosphate group by hydrolysis, e.g. bisphosphatase.

Choice B: Compare and contrast a feedback inhibitor to a product inhibitor. Give **one** example.

A feedback inhibitor is a compound later on in a pathway inhibiting a earlier enzyme. e.g. PFK via citrate or ATP, citrate synthase by succinyl coA.

A product inhibitor inhibits the enzyme that made it. e.g. pyruvate dehydrogenase, citrate synthase.

11. (12 pts) Please do **one** of the following choices (a well labeled diagram is a suitable answer):

Choice A: How does the oxidation of NADH in electron transport differ from the oxidation of $FADH_2$ in electron transport? Which complexes/processes are similar, which are different?

NADH is oxidized by complex I – electrons go on Q to form QH2 (4 pts)

FADH is oxidized in complex II – technically it shuttles electrons from its substrate to FeS centers, electrons go on Q to form QH_2 (4 pts).

Protons are pumped across the membrane in complex I, but not complex II (2 pts)

The rest of the pathway is the same QH_2 to Complex III to cytochrome C to complex IV to O_2. (2 pts)

Choice B: Briefly discuss how ATP is synthesized by ATP synthase. Describe the source of energy and the key features of the mechanism of the enzyme.

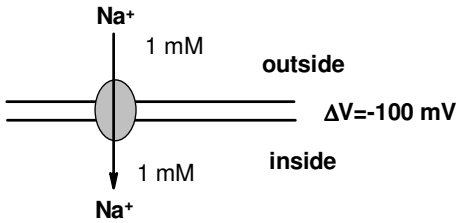
A high concentration of hydrogen ions across the inner membrane is the source of energy (4 pts)

When protons pass through ATP synthase the gamma subunit rotates (4 pts)

This rotation changes the conformation of b-subunits, in the following order (4 pts)

1: low affinity for ADP or ATP
2: high affinity for ADP + Pi so they bind.
3: ATP is more stable than ADP+Pi, so ATP forms spontaneously.
1: low affinity, so formed ATP is released.

12. (5 pts) A transmembrane enzyme catalyzes the movement of Na^+ ions from outside the cell to inside the cell. Assume that the concentration of Na^+ ions outside the cell is maintained at 1 mM, the concentration inside is 1 mM, and the membrane potential is -100 mV (inside negative). How many sodium ions would have to be transported to generate sufficient energy to convert ADP + P_i to ATP, assuming that this enzyme could couple sodium transport to ATP synthesis. *[Please use the back of the previous page for calculations if necessary.]*



$$\Delta G = RT \ln \frac{[X]_{in}}{[X]_{out}} + ZF\Delta V$$

$$= RT \ln \frac{0.001}{0.001} - (+1)(96000)(0.1)$$

$$= 0 - 9.6 kJ/mol$$

$$= -9.6 kJ/mol$$

One ATP requires ~30 kJ/mol therefore 4 Na would have to be *transported, providing 38.4 kJ/mol of energy.*

13. (16 pts) You are running in the Pittsburgh marathon next week and the morning of the race you have a pancake breakfast, a meal rich in carbohydrates. Please answer **all** parts *i – iv*. Note that *part iii* has choices.

i) Describe changes in the levels of ATP, AMP and glycogen in the liver that would occur as you are running the race. Do the levels of these compounds increase, decrease, or remain relatively constant (3 pts)?

- The liver's job is to produce glucose during the race.
- Glucose would be released from glycogen, so **glycogen levels must fall**.
- Glucose can also be produced from gluconeogenesis, this requires ATP, so **ATP would decrease somewhat, or stay nearly constant** since it can be replenished (for a while) by the formation of AMP using the following reaction: 2ADP → ATP + AMP.
- Consequently **AMP levels would rise**.
- Note that the liver cannot generate ATP by glycolysis during this period since F26P levels will be low and PFK-1 will be off – regardless of the ATP, ADP, or AMP levels.

ii) Which hormone would be released by the pancreas during the race? Why? (2 pts).

Glucagon (epinephrine was accepted, 1 pt) due to low blood sugar (1 pt).

iii) Please do **one** of the following three choices. Be sure to indicate your choice (8 pts).

Choice A: How would this hormone affect the regulation of glycogen synthesis/degradation. You should name the enzymes involved and clearly state how they are regulated and why they are regulated in this manner.

- Glucagon binds to its receptor and causes the phosphophorylation of enzymes by a signaling cascade (e.g. G-protein, adenyl cyclase, cAMP, activation of protein kinases.)
- Glycogen synthase will be inactivated by phosphorylation, preventing the storage of glucose in glycogen.
- Glycogen phosphorylase will be activated by phosphorylation, causing the release of glucose from glycogen, allowing the liver to provide glucose to the blood.

Choice B: How would this hormone affect the regulation of glucose oxidation (glycolysis) or glucose synthesis (gluconeogenesis)? You should name the enzymes involved and clearly state how they are regulated and why they are regulated in this manner.

- F26P levels would fall since they are proportional to blood glucose levels (the enzyme that degrades F26P is active when phosphorylated).
- PFK-1 in glycolysis requires F26P for activity, so glycolysis is off – the liver doesn't oxidize glucose under these conditions.
- Bisphosphatase-1 in gluconeogenesis is no longer inhibited, so glucose is made from pyruvate, until AMP levels get too high, and then it is shut off because there is insufficient ATP to make glucose (AMP inhibits bisphosphatase-1).

Choice C: In what way are the pathways of glucose oxidation (glycolysis) or glucose synthesis (gluconeogenesis) sensitive to the energy levels of the liver cell? You should name the enzymes involved and clearly state how they are regulated and why they are regulated in this manner.

- As AMP builds up, PFK in glycolysis will be activated as the liver tries to restore its ATP, but it can't do much since F26P levels are low, so the liver will not oxidize glucose since there is demand for glucose into the blood.
- High levels of AMP will inhibit bisphosphatase in gluconeogenesis, the liver won't make glucose since it doesn't have the ATP.

iv) Sometime during the marathon you "hit the wall" and have very little energy available and can't keep up your former pace (for me, this would be after about 1/2 mile). What types of compounds (e.g. carbohydrates, triglycerides, aminoacids) are now providing energy and what metabolic pathways are being used to generate this energy? (3 pts)

Your glycogen stores are gone, so fatty are oxidized by fatty acid oxidation. In very skinny people (no fat reserves – not me!) proteins would then be used leading to loss of muscle tissue. Without proper training marathons can be hazardous to your health.