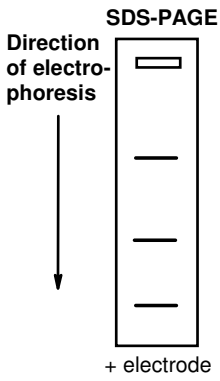
This six page exam contains 100 pts. Allot 1 min/2 pnts.

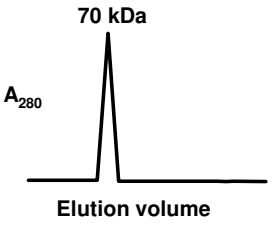
1. (5 pts) Please do **one** of the following three choices:

Choice A: In very general terms, how does the structure, or arrangement, of atoms in an object (e.g. a protein in a crystal) affect the scattering of X-rays.

Choice B: Briefly describe the final step in the determination of a structure by X-ray diffraction.

Choice C: A protein is denatured using sodium dodecyl sulfate (SDS). The protein is then run on an SDS-PAGE and the image of the gel is shown on the right. The protein is known to contain 5 kDa, 10 kDa, and 20 kDa subunits. The same protein, in its native state, is run on a Gel filtration column and the elution profile from that column is also shown on the right. Please answer the following two questions:





i) Label the bands on the SDS gel with the correct molecular weights (there are no standards on this gel, just the protein of interest)(1 pt).

ii) Give the quaternary structure that is consistent with both experiments. *Briefly* justify your answer (4 pts).

Choice A: The electrons scatter the x-rays (1 pt). The different positions of the atoms cause interference in the x-rays scattered from different atoms (4 pts), causing an increase or decrease in the intensity of the scattered X-rays.

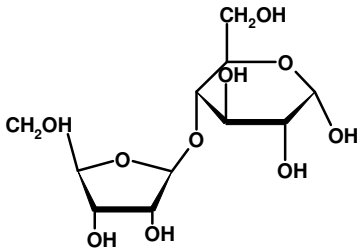
Choice B: Atoms are placed into the electron density such that there is a good match between the size of the electron density and the atoms that were placed into the density.

Choice C: Top band is 20 kDa, middle 10 kDa, lower, 5 kDa (-1 pt if order is reversed). The smallest molecular weight is 35 kDa. The native molecular weight is twice this, making the native form a heterohexamer, with two subunits of each size. ($\alpha_2\ \beta_2\ \gamma_2$), which gives the 70 kDa molecular weight observed in gel filration.

2. (5 pts)

i) Are these sugars **both aldoses**, both ketoses or one of each (1 pt).

ii)Which of the following is the correct name for the dissacharide shown on the right. Justify your answer if you want partial credit for an incorrect answer.



a) β-ribofuranosyl (1-4) β-glucopyranose **(+2, wrong chirality on glucose)**

b) β-fructofuranosyl (2-4) α-glucopyranose (0)

c) β-ribofuranosyl (1-4) α-glucopyranose (+4)

d) α-glucopyranosyl (4-1) β-ribofuranose (0)

e) β-ribofuranosyl (1-4) α-glucopyranoside (+3, since the only error is -oside versus -ose)

The reducing end is on the right. Therefore the first sugar is ribose followed by glucose. The anomeric carbon is in the β configuration on the ribose and it is linked to the 4th carbon on glucose, giving a (1-4 linkage). The configuration of the anomeric carbon on the glucose is α, so "C" is the correct answer.

3. (8 pts) Please do **one** of the following two choices:

Choice A: *Briefly* describe the chemical structure of bacterial cell walls.

Choice B: Compare and contrast the chemical structure of cellulose to glycogen (or starch). What is the normal biochemical role of cellulose and glycogen?

Choice A: Linear polymers of modified glucose: -NAM-NAG-NAM-NAG- **(4 pts)**

A peptide chain is attached to the NAM saccharide and this is crosslinked to the NAM residue on an adjacent linear polymer. **(4 pts)**

Choice B: Both are polymers of glucose. Cellulose has β(1-4) linkages and starch has α(1-4) and α(1-6) linkages, giving rise to a branched polymer **(6 pts, -1 if α(1-6) & branching is not discussed)**

Cellulose is a structural polysaccharide found in plants. **(+1)**

Glycogen is used to store glucose in the cell for energy storage. **(+1)**

4. (5 pts) *Briefly* describe the chemical difference between a phospholipid and a triglyceride. A simple sketch is an acceptable answer.

A triglyceride is composed of a glycerol molecule with attached three fatty acids using ester linkages.

A phospholipid replaces the fatty acid at position 1 with a phosphate group that may link to other groups (such as choline)

5. (10 pts) Please do any **one** of the following three questions:

Choice A: Briefly describe the role of the hydrophobic effect on the formation of phospholipid bilayers and micelles. Your answer should also discuss why these two compounds form structures with different shapes.

Choice B: Briefly explain why the mainchain conformation of membrane proteins is either α-helix or β-barrel.

Choice C: Describe the main types of transport proteins in membranes, provide **one** example.

Choice A: The non-polar acyl chains will order water in monomeric phospholipids and fatty acids. When these compounds self-assemble and bury the non-polar acyl chains, the water will be released, increasing the entropy of the system, which is favorable. **(-2 if no indication of how the hydrophobic effect causes structure formation.)**

Phospholipids form bilayers because the head group has the same area as the two acyl tails. **(+2 pts)**

Fatty acids form spherical micelles because the polar head group is larger than a single acyl tail, consequently they pack together like cones, which generates a sphere. **(+2 pts)**

Choice B: These two structures are the only ones that can satisfy all mainchain hydrogen bonds. When a protein inserts into the membrane it has to break H-bonds to water molecules. These cannot be reformed to the lipid since the lipid has no electronegative donors and acceptors.

Choice C:

Passive transporter - moves molecules from high concentration to low. e.g. glucose transportor.

Active transporter - using energy (ATP) to move molecules from a high concentration to a low concentration, e.g. ATP synthase, running backwards.

Signal transducer - binds a compound on one side of the membrane and transmits a signal to the other side of the membrane. e.g. hormone receptor.

(+3 for each general area, +1 for example)

6. (6 pts) Please answer **one** of the following two choices:

Choice A: What physically happens to a phosopholipid bilayer when it undergoes a gel-to-liquid crystalline phase transition? How does cholesterol affect this phase transition?

Choice B: How does the chemical structure of a phospholipid affect the temperature (T_M) of its phase transition? What molecular force or interaction is involved?

Choice A: The acyl chains go from a well packed solid to a disordered liquid-like state. The bilayer remains a bilayer **(4 pts).** Cholesterol makes this transition occur over a broader temperature range, overall making the membranes more fluid **(2 pts)**

Choice B:

Longer acyl chains raise the melting temperature **(+2 pts)**, increasing van der Waals interactions.

Cis double bonds lower the melting temperature by causing a kink in the hydrocarbon chain **(+2 pts)** , decreasing van der Waals interactions **(+2 pts)**.

7. (12 pts) Pick any **one** of the three food groups: i) proteins (amino acids), ii) carbohydrates (glucose), iii) fats (fatty acids), and list (or provide an overview drawing) **ALL** of the pathways involved in the *complete* conversion of the compound to CO_2 *and* ATP. You should include key intermediates in your answer and the location of the pathway in the cell. You do not need to list all compounds in all pathways.

+6 pts for having pathways stated in correct order.

+4 pts for cellular locations.

+2 pts for one or two "key" intermediates.

Amino Acids:

TCA Cycle → NADH → Electron transport → Proton gradient → ATP synthesis.

Glucose:

glycolysis → pyruvate → Acetyl CoA → TCA cycle→ NADH → Electron transport → Proton gradient → ATP synthesis

Fatty acids:

fatty acid oxidation → Acetyl CoA → TCA cycle→ NADH → Electron transport → Proton gradient → ATP synthesis

Glycolysis in the cytosol, Fatty acid oxidation & TCA cycle in the mito matix
Electron transport and ATP synthesis - inner membrane of mitochondria

8. (12 pts) Please do **one** of the following two choices:

Choice A: Briefly discuss how ATP is synthesized in the mitochondria. Your answer should include a brief discussion of why the overall change in the Gibbs free energy during this process is favorable and a description of the mechanism of ATP synthase.

Choice B: A three step biological pathway: A → B → C is found to be spontaneous, yet the difference in the standard energies between compounds A and B is large and positive. Briefly describe **both** of the possible types of coupling that can be used to make the step from A to B spontaneous. Provide ***one*** example.

Choice A:

- The higher concentration of protons on one side of the membrane results in a negative ΔG for transport of protons. The voltage difference across the membrane also contributes to the negative ΔG. $\Delta G = RT \ln [H_{IN}]/[H_{OUT}] + ZF\Delta V$ **(3 pts)**
- The ATP synthase contains 3 β-subunits whose conformation depends on the relative location of the gamma subunit.
- The three possible conformations of the β-subunit are such that one binds nothing, the second ADP+Pi, the third ATP. A single β-subunit cycles between these three due to rotation of the gamma subunit by 120 degrees each time 3 protons come through the ATP synthase. During a complete cycle of a 360 degree rotation, a single β-subunit will:
  1. bind ADP + Pi
  2. convert ADP + Pi to ATP
  3. release ATP

+9 pts for description of mechanism

Choice B:

Direct coupling occurs if the transition from A to B is directly coupled to a favorable reaction, such as phosphate transfer from ATP on the same enzyme. The Gibbs energy can become negative because the overall standard energy is reduced. Example, hexosekinase, PFK.

Indirect coupling occurs if the step from B to C is very favorable, such that the concentration of B is less that its equilibrium concentration. The Gibbs energy is negative in this case because ln[B]/[A] will be negative. e.g. aldolase, malate dehydrogenase.

9. (8 pts) Please do one of the following two choices. Please indicate your choice.

Choice A:

i) What enzymatic reaction is catalyzed by a *protein* kinase (4 pts)?

ii) What is the importance of reactions of this type in regulation of metabolism (4 pts)?

Choice B:

Compare and contrast a feedback inhibitor to a product inhibitor (6 pts). Provide an example of *either* and indicate the biological importance of the regulation in your example (2 pt)

Choice A:

i) The transfer of a phosphate group from ATP to an -OH group (Ser, Thr, Tyr) on a protein.

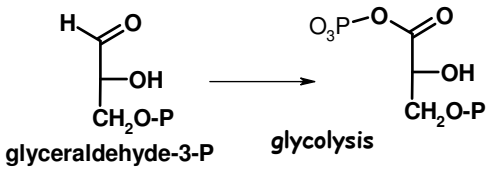
ii) The addition of a phosphate can cause an allosteric change, activating or inhibiting the enzyme.

Choice B:

A feedback inhibitor is a compound that inhibits a previous step in the pathway, excluding the step that made the compound. e.g. High levels of citrate turn off glycolysis by inhibiting PFK. High levels of citrate suggest that the TCA cycle does not need any additional acetyl CoA.

A product inhibitor is inhibition of an enzyme by its own product. Example is pyruvate dehydrogenase, which is inhibited by acetyl-CoA. If the levels of acetyl-CoA are high, there is no need to generate more.

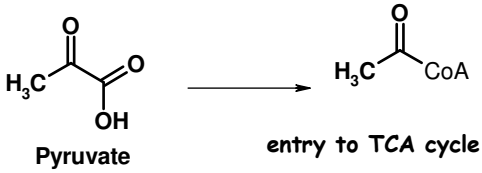
10. (10 pts) Pick *any one* oxidation step in *any* biochemical pathway and answer all of the following questions. *(The structures on the right may be helpful.)*



glyceraldehyde-3-P → glycolysis

i) List the pathway(s) that your reaction is found (2 pt).

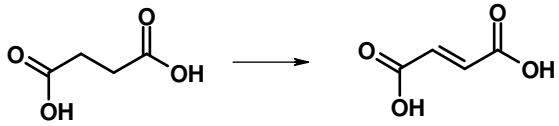
(see diagram)



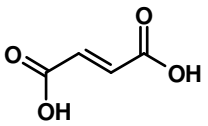
Pyruvate → entry to TCA cycle

ii) Draw the structure of the reactant and product, if the reactant is shown on the right, just draw the product (2 pts).

(see diagram)



Succinate → TCA cycle
similar reaction in fatty acid cycle

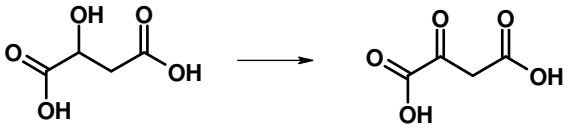


Fumarate

This is converted to malate by the addition of water, NOT an oxidation

iii) Name the enzyme that catalyzes the reaction (1 pt).

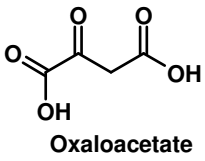
dehydrogenase



Malate → TCA cycle
similar reaction in fatty acid cycle

iv) Indicate any cofactors or cosubstrates that would be required (1 pt).

NAD^+ or FAD, as electron acceptors.



Oxaloacetate

This cannot be oxidized any further

v) Show, by balancing, that the reaction you have selected is indeed an oxidation (2 pts).

All are 2 electron redox reactions

vi) In *very* general terms, what is the most important product from *any* oxidation (2 pts).

Energy, in the form of NADH or $FADH_2$

Loss of electrons also accepted.

11. (5 pts) Please do one of the following two choices:

Choice A: Although glycolysis does not directly utilize O_2, it will quickly stop in the absence of oxygen unless anaerobic metabolism occurs. What is the purpose of anaerobic metabolism? What product of commercial value can be produced by anaerobic metabolism in yeast?

Choice B: Why can't humans convert excess calories from ingested fat into sugar?

Choice A:

- Anaerobic metabolism regenerates NAD^+ to allow glycolysis to continue. Otherwise all of the NAD^+ will be used up because the NADH is not oxidized by electron transport.
- Anaerobic metabolism in yeast produces ethanol.

Choice B:

- The carbon in fatty acids ends up as acetyl CoA.
- The step from pyruvate to acetyl CoA is irreversible in humans, therefore the carbon in acetyl CoA cannot be used to produce pyruvate which could be used, via gluconeogenesis, to produce glucose.

12. (14 pts) Pick ***any*** coordinately regulated step in glucogen or glucose metabolism and briefly describe *(use the back of the previous page if you need additional room)*:

i) How it is regulated, including a brief description of either direct or indirect hormonal control. **(8 pts for a mostly complete description).**

ii) The biological importance of this regulation. **(+6 pts)**

i) Energy sensing of glycolysis by PFK.

- PFK is in a pathway that generates energy (ATP)
- High levels of ATP should inhibit PFK since it does not make sense to make more ATP.
- High levels of AMP and ADP, which indicate low levels of ATP, should turn on PFK, because the cell needs more ATP.

ii) Hormonal control of glycolysis by F26P levels.

- Under condition of high blood sugar, the hormone insulin binds to its receptor, causing dephosphorylation of enzymes.
- The dephosphorylation causes F26P levels to rise.
- F26P activates PFK in glycolysis, allowing the excess glucose to become oxidized if ATP is needed.

iii) Hormonal control of gluconeogenesis by F26P levels.

- Under condition of low blood sugar, the hormone glucagon binds to its receptor, causing phosphorylation of enzymes due to G-protein activation of adenyl-cyclase.
- The phosphorylation causes F26P levels to fall.
- PFK in glycolysis requires F26P for activity, so glycolysis is off.
- F16P bisphosphatase become active with low levels of F26P, allowing the production of glucose for export out of the cell.

iv) Hormonal control of glycogen synthesis/degradation.

- Under condition of high blood sugar, the hormone insulin binds to its receptor, causing dephosphorylation of enzymes. This dephosphorylated form of glycogen synthase is active, causing the excess glucose to be stored as glycogen.
- Under condition of low blood sugar, the hormone glucagon binds to its receptor, causing phosphorylation of enzymes due to G-protein activation of adenyl-cyclase. The phosphorylated form of glycogen phosphorylase is active, releasing glucose from glycogen.