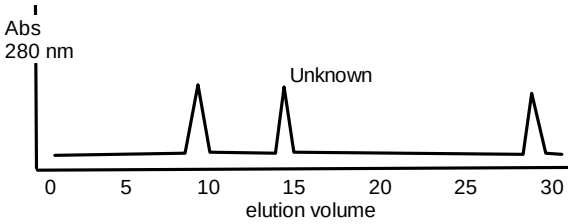
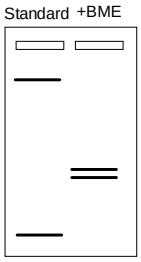
Instructions: This exam consists of 100 points on 6 pages. Please use the space provided to answer the question, or the back of the preceding page. In questions with choices, unless otherwise indicated all your answers will be graded and you will receive the best grade. Allot 1 min/2 points.

1. (5 pts) What fundamental principle allows one to determine the structure of proteins using X-ray diffraction? Name the principle and give a brief description.

X-rays are scattered from electrons in atoms.
The relative position of atoms affects the interference between scattered X-rays
This affects the intensity of the scattered X-rays, i.e. constructive or destructive interference
The intensities (and phase) can be used to deduce the position of the atoms.

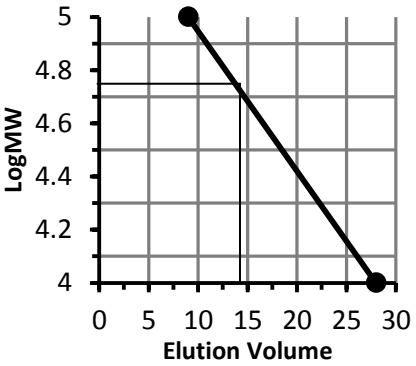
2. (6 pts) The elution profile for gel filtration (size exclusion) chromatography is shown to the right. The standard molecular weights are 10,000 and 100,000 Daltons. The unknown peak is the middle peak. A useful plot is shown below the elution profile. (log30,000=4.48, log60,000=4.78, log90,000=4.95)





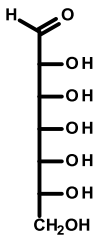
The SDS-PAGE gel (without β-mercaptoethanol) shows two bands of equal intensity with molecular weights of 14,500 (α chain) and 15,500 (β chain).

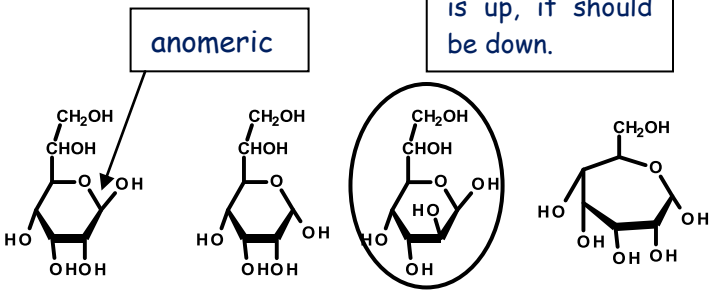
Determine the quaternary structure of this protein. *You **must** briefly justify your answer for full credit.*



The minimum quaternary structure is αβ since the bands on the SDS-gel are equal in intensity. The predicted molecular weight for αβ is 30,000. The native molecule weight is 60,000 – the elution volume of ~14 corresponds to logMW ~ 4.7. Therefore the quaternary structure is $(\alpha\beta)_2$.

3. (5 pts) The following shows the linear form of a **seven** carbon monosaccharide (left side) and several choices for the ring form. Please answer all of the following parts of the question.





Incorrect because 2nd OH is up, it should be down.

This is a possible ring form, not the most stable, but possible.

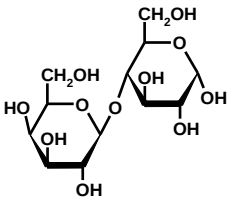
i) Is this an aldose or a ketose (circle correct word) (2 pt)
ii) Circle the **one** circular form that could **not** be generated from the linear sugar (2 pts)
iii) Label the anomeric carbon on the first circular form (1 pt).

4. (4 pts) Fill in the blanks. The _anomeric_____ carbon is always involved in the formation of the ___glycosidic __ bond that joins two monosaccharides to form a disaccharide. A common disaccharide is lactose_____, and it is composed of monosaccharides __galactose _____ and glucose. (or Sucrose, composed of glucose and fructose).

5. (3 pts) Fill in the six blanks to complete the name of this sugar:

β_-galacto pyranosyl -(1 → 4)- _a__ _glucopyranose

1 2 3 4 5 6



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

Choice A: Compare and contrast the structure **and** biological function of glycogen and cellulose. In what ways are they similar, and in what ways do they differ?

Choice B: Bacterial cell walls contain a polysaccharide component that is similar to cellulose.

i) In what ways does the polysaccharide component in bacterial cell walls differ from cellulose?

ii) What additional component is found in bacterial cell walls that are not found in cellulose?

Choice A:

- Both are polymers containing glucose (2 pts)
- In glycogen the linkages are linear α(1-4) with α(1-6) branches. In cellulose they are β(1-4) linkages with no branching (2 pts)
- Glycogen is used for glucose storage, cellulose is a structural polysaccharide. (2 pts)

Choice B:

- The glucose units are replaced by alternating NAG and NAM residues – these are modified glucose, cellulose consists of only glucose. (3 pts)
- The NAM units in the carbohydrate portion of each chain are linked together via a peptide (3 pts).

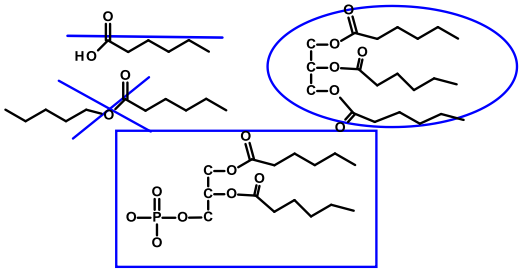
7. (4 pts) Four compounds (A-D) are shown on the right.

i) **Circle** the triglyceride,

ii) Put a **box** around the phospholipid,

iii) Draw an **'X'** through the wax.

iv) Draw a horizontal line (---) through the fatty acid.



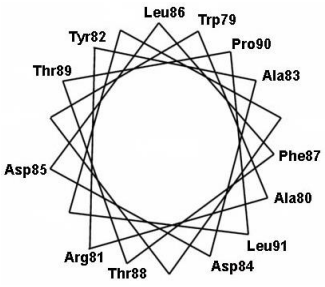
8. (6 pts) Please do **one** of the following choices.

Choice A: What is the critical micelle concentration (CMC)? How does it depend on the number of carbons?

Choice B: How does cholesterol affect the properties of membranes and why is this effect important for function?

Choice C: In what way is the function of membrane transport proteins similar to soluble enzymes? In what ways do they differ?

Choice D: The diagram on the right is a helical wheel depiction of an α-helix in a membrane protein. Indicate which part of this helix is exposed to the lipids and what energetic features would stabilize this interaction? Briefly justify your answer.



Choice E: Explain why the potassium channel is selective for K^+ ions.

Choice A: It is the highest possible concentration of monomeric fatty acids in solution. Above this concentration any added fatty acids will form micelles. As the fatty acid length increases the CMC should decrease because the solubility will decrease.

Choice B: Cholesterol keeps the membrane fluid, permitting conformational changes to occur in membrane enzymes and the diffusion of electron carriers, such as CoQ.

Choice C: Both convert substrate to product. A soluble enzyme will typically cause a change in the chemical structure of the substrate. The transport protein doesn't change the structure, but binds the "substrate" on one side of the membrane and moves the compound across the membrane, producing the product. The only difference between substrate and product is the location.

Choice D: The right side is exposed to the lipid because it contains mostly non-polar residues. These will interact favorable with the lipids due to the hydrophobic effect and van der Waals.

Choice E: The ion must be desolvated to cross the channel. The energetically unfavorable loss of water is compensated by the favorable interaction between the K+ ion and the C=O groups in the selectivity filter. The size of the selectivity filter is just right for K+, maximizing electrostatic interactions.

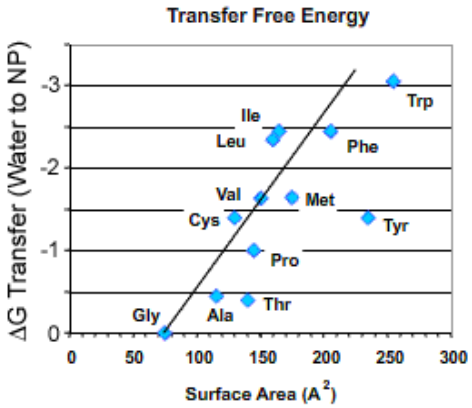
9. (6 pts) What fundamental thermodynamic interaction is responsible for the spontaneous *assembly* of both fatty acid micelles and phospholipid bilayers? [Hint: It is entropic]. In what way does this interaction affect the final structure of the micelle **or** bilayer?

- The hydrophobic effect 4 pts and then 2 pts for the following:
- Free fatty acids or phospholipids have extensive non-polar acyl chains that order water.
- When micelles/bilayers form the acyl chains interact with themselves and are buried from the water.
- The released water has an increase in entropy, which is favorable.

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

Choice A: Olive oil is a triglyceride that melts at 10°C and is therefore a liquid at room temperature. Coconut oil is a triglyceride that melts at 25°C and is therefore a solid at room temperature. What is the most likely difference between the fatty acids in these two oils and how does this difference affect their melting temperatures? What fundamental thermodynamic interaction (e.g. H-bonds, electrostatics) is responsible for this difference?

Choice B: The concentration of two short polypeptides in membranes is measured. One polypeptide, consisting entirely of alanine residues, is predominately found in the aqueous solution, while the other peptide, which consists of phenylalanine residues, is predominately found dissolved in the membrane. Explain, in **quantitative** terms, the different behavior of the two peptides. The free energy of transferring a Gly reside from water to a non-polar substance is +1 kcal/mol. The free energy for the transfer of sidechains is given in the diagram on the right.



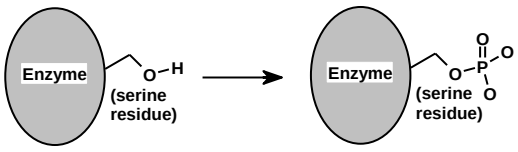
Choice A:

Olive oil contains cis unsaturated double bonds that cause a bend in the fatty acid chain, disrupting **van der Waals** interactions. Coconut oil has fewer cis double bonds and therefore more van der Waals interactions, raising the melting temperature.

Choice B:

- The overall standard energy (ΔG^o) for insertion of the peptide consists of a positive term for the mainchain atoms (hindering insertion) (+ 2 pts).
- This is offset by a negative contribution from the side chain. Alanine residues have a negative standard energy of -0.5. Since these don't insert, the contribution of the mainchain atoms has to be at least +0.5 (+4 pts)
- Phenylalanine sidechains have a larger favorable standard energy for transfer, this is sufficient to overcome the positive standard energy for the mainchain atoms. (+ 4 pts)

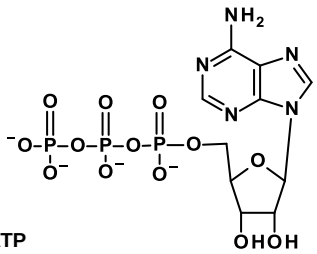
11. (7 pts) Is the following reaction catalyzed by a kinase or a phosphatase (2 pts)? Briefly justify your answer and list any reactants or products that may be missing from the diagram (2 pts). How does the phosphorylation of enzymes in this manner affect their activity (3 pts)?



- The reaction shown is catalyzed by a kinase, which transfers a phosphate group from ATP to the substrate. (2 pts)
- The missing reactant is ATP, and the missing product is ADP. (2 pt)
- The phosphorylation will cause an allosteric change that may activate or inhibit the enzyme (3 pts).

12. (3 pts) Why is ATP a "high energy compound"? That is, why does the hydrolysis of ATP to form ADP and P_i release energy (the structure of ATP is shown on the right).

The negative charges on the phosphate will repel each other, this electrostatic interaction will raise the energy of ATP. When the phosphate is released, this repulsion is removed.



ATP
(adenosine triphospate)

13. (2 pts) The maximum yield of ethanol from glucose will be obtained under conditions of high or low oxygen content? (circle correct answer). [Under conditions of low oxygen, NAD^+ cannot be regenerated by electron transport. The conversion of pyruvate to ethanol oxidizes NADH back to NAD^+.]

14. (2 pts) True or false (circle correct answer)? When glucose is converted to ethanol most of the energy is lost in the process. [Glucose contains 38 ATP units of energy. Glycolysis only releases 2 ATP units, so most of the energy is still contained in the ethanol.]

15. (12 pts) Assume that you had a cream cheese bagel for breakfast.

i) Briefly discuss how the carbon atoms in the food are ultimately converted to CO_2 for **either** fats **or** carbohydrates **or** amino acids that were contained in the bagel. You only need to:

a) state the major pathways that would be involved in the production of CO_2,
b) input and output compounds of that pathway,
c) the cellular location of each pathway.

ii) Briefly describe the flow of energy as the carbohydrate, fat, or protein is metabolized, i.e. how is the energy that is released by oxidative steps in each pathway is ultimately converted to ATP (i.e. briefly discuss electron transport and ATP synthesis). *Your description of electron transport should list each stage of electron transfer. There is no need to discuss the mechanism of ATP synthesis in detail* (6 pts).

i) 4 pts for:

Triglycerides → fatty acids → Activation (cytosol) → oxidation (M. Matrix) →TCA (M. Matrix).

Carbohydrates: Glucose → glycolysis (cytosol) →TCA (M. matrix).

Amino acids: enter TCA (M. matrix).

All carbon from the above processes is released as CO_2 in the TCA cycle (+2 pts)

ii) Energy from oxidation of carbon is stored on NADH or $FADH_2$ (2 pts)

In electron transport, the electrons flow as follows: (3 pts)

NADH: Complex I →CoQ→Complex III→Cytochrome C→Complex IV→O_2.
$FADH_2$: Complex II →CoQ→Complex III→Cytochrome C→Complex IV→O_2.

Protons are pumped across the membrane in complexes I, III, IV. (1/2 pt)
NADH = 10 H^+
$FADH_2$ = 6 H^+

The protons flow back through ATP synthase, which uses the energy in the proton gradient to make ATP. $3H^+$ ~ 1 ATP (1/2 pt)

16. (9 pts) All metabolic pathways, when operating, show a negative Gibbs free energy (ΔG) for each step.
i) What does the sign of the Gibbs energy indicate about each step in the pathway (3 pts)?
ii) Many steps in pathways have an unfavorable standard energy change (ΔG^o), for example: a) the conversion of fructose-6-P + $P_i \rightarrow$ fructose-1,6-P in glycolysis, b)aldolase in glycolysis, c) the formation of citrate in the citric acid cycle. Briefly describe **one** general mechanism for converting the positive standard energy change to a negative Gibbs free energy (6 pts). The use of the following formula may be useful in illustrating your answer: $\Delta G = \Delta G^o + RT \ln [B]/[A]$.

i) The reaction (pathway) will be spontaneous in the forward direction. (3 pts)

ii) (6 pts)

Direct coupling (e.g. kinase). Couple the reaction to the hydrolysis of ATP by direct transfer of the phosphate from ATP to the compound. This changes ΔG^o, making it negative.

Indirect coupling. A step in the pathway (usually the last one) has a large energy drop. This keeps the concentration of all intermediates prior to the step below their equilibrium concentration. This makes the ln[B]/[A] term negative.

17. (4 pts) Please do **one** of the following **two** choices

Choice A: Complete **one** (only one will be graded) of the following fill in the blanks.

i) Prior to entering the _____TCA_____ pathway, pyruvate is converted to _____AcetylCoA____, using _____NAD+_____ as the electron acceptor. This reaction is catalyzed by a _dehydrogenase_ (general name).

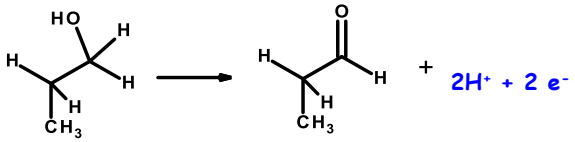
ii) In the _TCA/fatty acid oxidation_ pathway, an alkane is converted to a __alkene_____, using ___FAD_____ as the electron acceptor. This reaction is catalyzed by a _dehydrogenase_ (general name).

iii) In the _TCA/fatty acid oxidation_ pathway, an alcohol is converted to a __ketone_____, using ___NAD+___ as the electron acceptor. This reaction is catalyzed by a _dehydrogenase_ (general name).

iv) In the _glycolysis_ pathway, an aldehyde is converted to a _phosphate ester/carboxylic acid_, using _____NAD+_____ as the electron acceptor. This reaction is catalyzed by a _dehydrogenase__ (general name).

Choice B:

i) Balance the redox reaction on the right, is it an oxidation or a reduction?



The oxygen atoms are balanced already.
The left compound has 8 H, the right 6.
Two hydrogen atoms have to be added to the right side, indicating that the alcohol lost electrons ($H^+ + e^-$) and was therefore oxidized.

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

18. (6 pts) Please do **one** of the following choices.

Choice A: Glycolysis, gluconeogenesis, and the TCA cycle are all regulated by "energy sensing". Select **one** of the three pathways and describe:

i) the step that is regulated (1 pt).

ii) the compounds that regulate that step, and whether they activate or inhibit the step (4 pts).

iii) why this regulation is useful to the cell (1 pts).

Choice B: The liver cell responds to a number of different hormones, including insulin, glucagon, and epinephrine. Select any **one** of these hormones and:

i) State under what conditions the hormone would be released (e.g. low blood glucose levels) (1 pt).

ii) Whether proteins become phosphorylated, or not (1 pt).

iii) How the response to the hormone affects the synthesis and degradation of glycogen and why this response is appropriate for the needs of the organism (4 pts).

Choice C: Discuss how glycolysis and gluconeogenesis are subject to regulation by hormones. Your answer should indicate why this form of regulation is useful to the cell. [Hint: how are levels of F26P affected?]

Choice A:

i) Glycolysis: PFK – Inhibited by ATP, activated by AMP & ADP (allosteric). Since glycolysis produces ATP, high levels should shut it off. AMP and ADP levels indicate that the cell needs ATP, so glycolysis should be on.

ii) Gluconeogenesis: bis phosphatase – Inhibited by AMP. Since gluconeogenesis requires ATP (anabolic pathway), it should not operate unless there is sufficient ATP. Insufficient ATP would be indicated by high levels of AMP, turning the pathway off.

iii) TCA Cycle: Pyruvate dehydrogenase and citrate synthase are inhibited by ATP and NADH. High levels of these compounds indicate that the cell has enough energy, so the entry of carbon into the TCA cycle should be suppressed because the TCA cycle generates energy (ATP/NADH).

Choice B:

Glucagon or epinephrine released - Low blood glucose/glucose demand:

- Enzymes become phosphorylated (due to activation of G-proteins and adenyl cyclase.)
- Glycogen phosphorylase is active, releasing glucose from glycogen.
- This allows the liver to provide glucose to raise the blood glucose levels.

Insulin released - High blood glucose

- Binding of insulin to its receptors leads to dephosphorylation of enzymes
- Glycogen synthase is active when dephosphorylated, so glucose is incorporated into glycogen
- This allows the liver to store the excess glucose in glycogen.

Choice C:

- F2,6 P levels are regulated by hormones due to the fact that the enzymes that make (PFK-2) and degrade (bisphosphatase 2) are phosphorylated/dephosphorylated.
- High blood sugar – F2,6 P levels will be high.
- High F26P levels activate PFK in glycolysis, this makes sense because there is plenty of glucose so glycolysis should be allowed to be on in case the liver cell needs to make ATP.
- High F26P levels inhibit the bisphosphatase in gluconeogenesis, so only one pathway is on.
- Low blood sugar – F2,6 P levels will be low, glycolysis will be off, but gluconeogenesis will be on.

Bonus Questions (1 ½ pts each)

1. We possess an enzyme (lysozyme) that can digest bacterial cell walls, why is it not possible for us to digest cellulose (you need to briefly explain why lysozyme cannot digest cellulose).

Lysozyme specifically recognizes the N-acetyl group on NAM/NAG units. This group is not present on cellulose.

2. Briefly explain why a "high carb diet" is not necessarily the best for marathon runners.

Muscle glycogen stores will be depleted during the race (called hitting the wall), so you need some reserves of fat.

3. What enzyme do lactose intolerant individuals lack? What does this enzyme do?

The enzyme is lactase and it breaks down lactose to galactose and glucose allow the individual to utilize the sugar instead of bacteria.

4. Why does the consumption of high fructose corn syrup potentially lead to an increase in body fat? Why type of syrup would be less likely to cause this problem (Oh Canada!)?

Fructose enters metabolism below the major control point, thus it is not well regulated. Excess calories in fructose will go to acetyl CoA and then the only way to store this carbon (in humans) is in fat.

Maple syrup is largely glucose which is tightly regulated since it is above PFK. Excess glucose will be stored in glycogen and not be