03-232 Biochemistry F2019 Exam 3 Name:_____

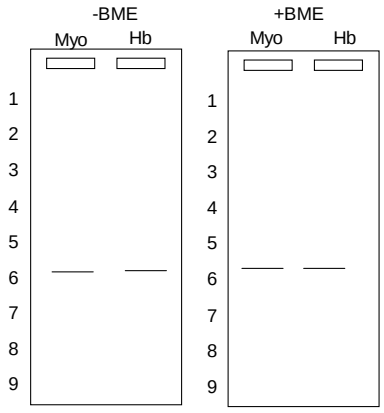
Instructions. This exam consists of 6 pages and 16 questions. On questions with choices all of your answers will be graded and you will receive the highest grade. Allot approximately 1 min/2 pts.

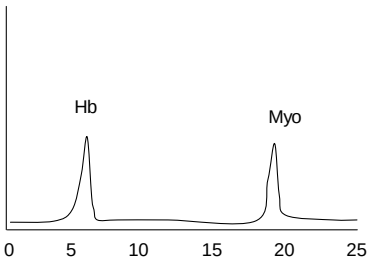
1. (9 pts) Myoglobin contains one polypeptide chain while hemoglobin consists of four chains, each of which is equal in size to myoglobin. There are no disulfide bonds in hemoglobin. Compare and contrast the results for the following three experiments. Feel free to use the images on the right to help illustrate your answer.

   i) SDS-PAGE, no β-mercaptoethanol (BME)

   ii) SDS-PAGE, with BME

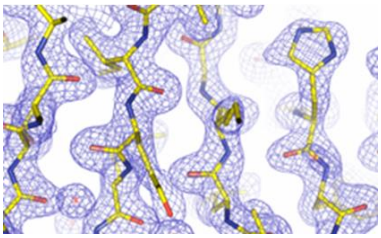
   iii) Size exclusion chromatography.





- The SDS-PAGE image will be the same with and without BME since there are no disulfide bonds.
- In both cases (hemoglobin and myoglobin) the SDS will denature the protein and there will be a single band for both proteins because hemoglobin's four chains are similar in size.
- In the case of size exclusion, there would be two peaks, the first peak is the hemoglobin since it is larger and cannot enter the beads. The myoglobin will be the second peak. The proteins are not denatured by this technique.

2. (6 pts)
i) What is the image on the right represent?
ii) Describe how was it obtained, beginning from a solution of pure protein?



i) This shows a fitted electron density map.
ii) It was obtained by:
1. Forming crystals of the protein
2. Collecting x-ray diffraction data
3. Calculating the map using intensity and phase information
4. Fitting the atoms into the density.

3. (3 pts) How is the anomeric carbon generated in carbohydrates? What are its properties?

It is the carbon that was a carbonyl (C=O) group. Either an aldehyde in aldoses (e.g. glucose) or a ketone in ketoses (e.g. fructose). When the sugar forms a ring, the C=O becomes an alcohol and goes from an achiral center to a chiral center.

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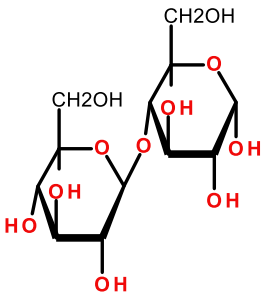
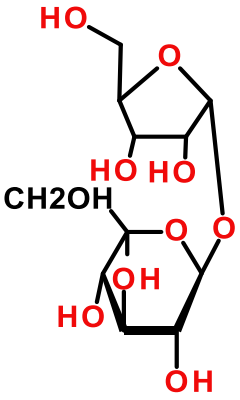
4. (6 pts) Please do one of the following choices:

Choice A: Name the sugar shown on the right.



B-glucopyranosyl (1-4) a- glucopyranose

Choice B: Draw the following sugar: β-glucopyranosyl (1-1) α-ribofuranoside.



5. (6 pts) Compare the following properties of **either** cellulose **or** glycogen to bacterial peptidoglycan:

i) The monomeric units

ii) How they are linked together.

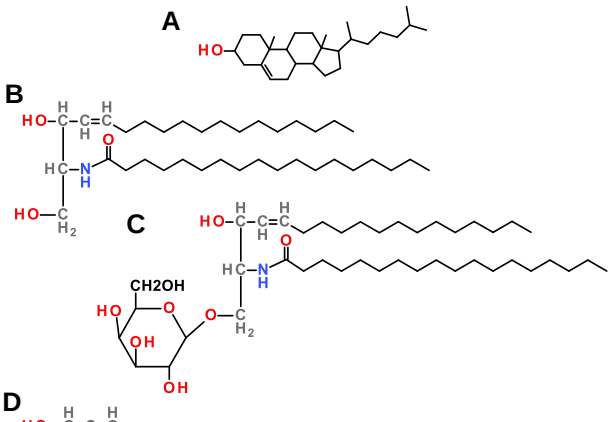
Cellulose and glycogen both are polymers of glucose, linked by beta(1-4) or alpha (1-4)+alpha (1-6), respectively.

The bacterial peptidoglycan is alternating NAG (N-acetyl glucosamine)/NAM (N-acetylmuramic acid), linked in linear beta (1-4) linkages. There are peptide linkages between the NAM units on adjacent polysaccharides.

6. (2 pts) The diagram on the right shows a number of molecules, labeled A-G. Write the correct letter next to the following names:

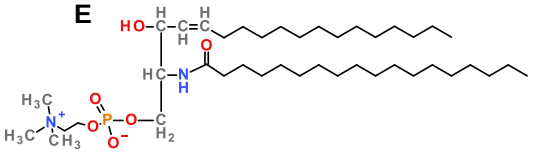
Sphingomylin __E___

Cholesterol ___A_____



B is ceramide, C is cerebroside, D is sphingosine

7. (6 pts) Draw any phospholipid you like and give its correct name. ***At least one of the fatty acids should be oleic acid (18:1 cΔ9).***



4 pts for overall correct structure

1 pt for correct name (1/2 head, 1/2 fatty acids)

1 pt for correct oleic acid.

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

Choice A: Why is cholesterol a critical component of mammalian cell membranes. In particular, in the inner mitochondrial membrane.

Choice B: What is the relationship between the melting temperature for the gel to liquid crystalline transition in lipids and the chemical structure of the acyl chain on the phospholipid.

Choice C: What is the critical micelle concentration (CMC) and how is it affected by the structure of the lipid.

Choice A: It makes membranes fluid, allowing for diffusion of electron carriers (Q)

Choice B: Depends on van der Waals, longer chains – higher melting, cis double bond causes kink in chain, reducing van der waals and the melting temperature.

Choice C: It is the concentration of a lipid where micelles begin to form. Below that concentration there is a true solution. To form micelles the polar head has to be larger than the non-polar tail and the CMC decreases as the amount of non-polar group increases.

9. (8 pts) Please do **one** of the following choices:

Choice A: Explain why the K^+ channel is selective for K^+ and will not allow smaller Na^+ ions to pass through.

Choice B: Both Alanine ($-CH_3$) and Valine ($-CH_2-(CH_3)_2$) are non-polar, yet polyalanine peptides will not insert into membranes while polyvaline peptides will. Explain this difference.

Choice A:
The channel is too small for hydrated ions, regardless of the type.
In order to fit through the channel it is necessary to dehydrate the ion. This is energetically unfavorable so the ion has to have favorable interactions with the C=O groups in the channel. This interaction is optimized for K ions, so the energy cost for dehydration is compensated by the K C=O interaction.

Choice B:
The polar atoms in the mainchain have more favorable van der Waals interactions with the polar solvent than they do with the non-polar lipids (this is not referring to hydrogen bonds, that is a separate issue). Consequently, it is unfavorable to move mainchain atoms from water to the lipid.
It is only favorable when the hydrophobic effect is sufficiently large to counter this effect. This is the case for valine, but not alanine.

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10. (10 pts) What is the difference between direct and indirect coupling. Give one example of each to illustrate your answer.

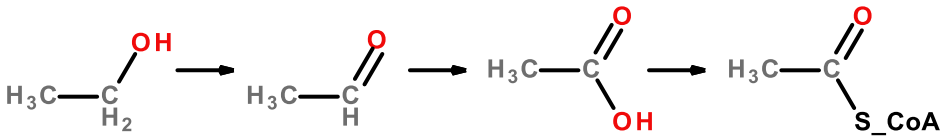
+4 for correct description, +1 for example.

Direct coupling - the energy is provided when the reaction occurs, in the active site of the enzyme. e.g. phosphate transfer in a kinase.

Indirect coupling – the concentration of the product is kept below its equilibrium concentration by a favorable reaction downstream, e.g. pyruvate kinase in glycolysis. This makes the ln[B]/[A] term in the Gibbs energy negative.

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

Choice A: In the metabolism of ethanol, the following series of reactions occur. Please answer the following questions.



i) What additional substrates are missing from the diagram? Why?

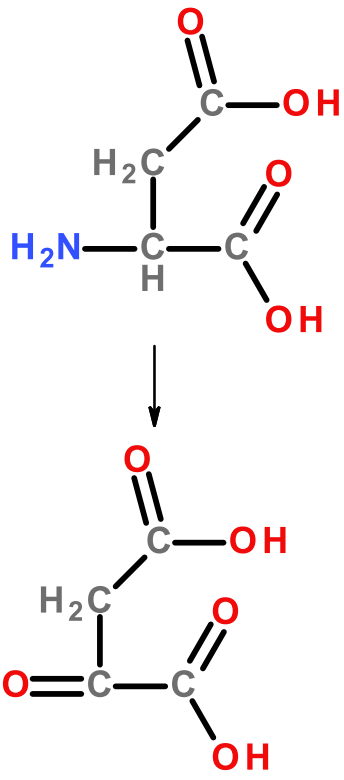
ii) What metabolic pathway will the last compound enter?

i) The first two reactions are redox (2 electron oxidations), so an electron acceptor is missing, NADH in both cases (FADH2 was accepted as an answer).
ii) The last compound is acetyl CoA, it enters the TCA cycle, condensing with oxaloacetate to form citrate.

Choice B: Please answer both of these (Hint: See Question 16, pg 6).

i) Explain how the amino acid aspartic acid would be fully oxidized to CO_2.

ii) Explain how the amino acid aspartic acid could be converted to glucose.



i:

- A transamination reaction converts the amino to a keto group, generating oxaloacetate, the last compound in the TCA cycle.
- This contains four carbons so these would be released as CO_2 after two turns of the cycle.

ii:

- Oxaloacetate is also generated from pyruvate in gluconeogenesis, which is then converted to phosphoenol pyruvate that is converted to glucose.

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12. (6 pts) In a resting neuron the membrane potential is -70 mV (inside negative) . The Na-K ATPase uses ATP hydrolysis to move 3 Na^+ ions across the membrane, from the inside to the outside. If the concentration of sodium is 10 mM inside the neuron, show that the concentration outside the cell is 37 mM, assuming that all the energy from ATP hydrolysis is available for ion transport. Assume RT = 2.5 kJ/mol.

How the value of 0.037 M on the outside was calculated (not required for your answer):

The energy associated with the Na gradient is:

$\Delta G = nFV + RT \ln [Na_{in}]/[Na_{out}]$

Since one ATP (30 kJ/mol) pumps 3 Na, the energy for each sodium is 10 kJ/mol.

The energy associated with the voltage gradient is (+1)(96)(0.07) = 6.72 kJ/mol

Therefore, the additional energy is 3.3 from the concentration gradient.

$3.28 = 2.5 * \ln [(0.01)/Na]$

$1.31 = \ln [(0.1)/Na]$

$e^{1.32} = 0.1/Na$ [Na] = 0.037 M

Expected answer:

$\Delta G = (+1)(96)(-.070) + 2.5 \ln (0.01/0.037)$

$=-6.7 -3.3= -10$ kJ/mol

Each sodium requires +10 kJ/mol to move to the outside of the cell, therefore 3 require +30, the energy released by ATP hydrolysis.

13. (4 pts) What property of Gibbs free energy is common to all pathways and what is the significance of this?

Negative for each step of the pathway, so that each step is spontaneous.

14. (8 pts) Please do **one** of the following choices:

Choice A: At Tarnival you ate way too much cotton candy (e.g. glucose) than you should have. Briefly describe the steps in hormonal regulation of glycogen metabolism, e.g. which hormone is released, are proteins phosphorylated or not, which enzyme is active, glycogen synthase or glycogen phosphorylase.

Choice B: Under what conditions (high/low blood glucose) is the liver cell is "allowed" to undergo glycolysis? Briefly discuss the regulatory events.

Choice C: Explain, at the molecular level, how PFK-1 in glycolysis is regulated by adenosine nucleotides.

***Choice A*:**

1. High blood sugar – insulin released
2. Receptor binding directly activates a tyrosine kinase. Subsequent signaling activates protein **phosphatases**, many enzymes lose their phosphate groups.
3. Glucose stored in glycogen - Glycogen synthase is active when dephosphorylated, glycogen phosphorylase is inactive

***Choice B*:**

- Glycolysis is only permitted when blood glucose is high. This leads to dephosphorylation and activation of PFK-2 that synthesizes F26P, therefore the levels of F26P rise.
- F26P is absolutely required for activation of PFK in glycolysis.

***Choice C*:**

- PFK-1 in glycolysis has two adenosine nucleotide binding sites. One site is the active site and this binds ATP which is the phosphate donor for F6P.
- The second site binds AMP, ADP or ATP. The phosphate group projects into the protein, thus the binding of ATP will lead to allosteric changes inactivating the enzyme. AMP and ADP have fewer phosphates so they will not cause the allosteric change and will cause PFK to be in the relaxed form.

15. (2 pts) In what way is the metabolism of triglycerides similar to that of glycogen?

Conversion to fatty acids occurs in the cytoplasm and the activity of lipases are controlled by hormones.

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16.(6 pts)

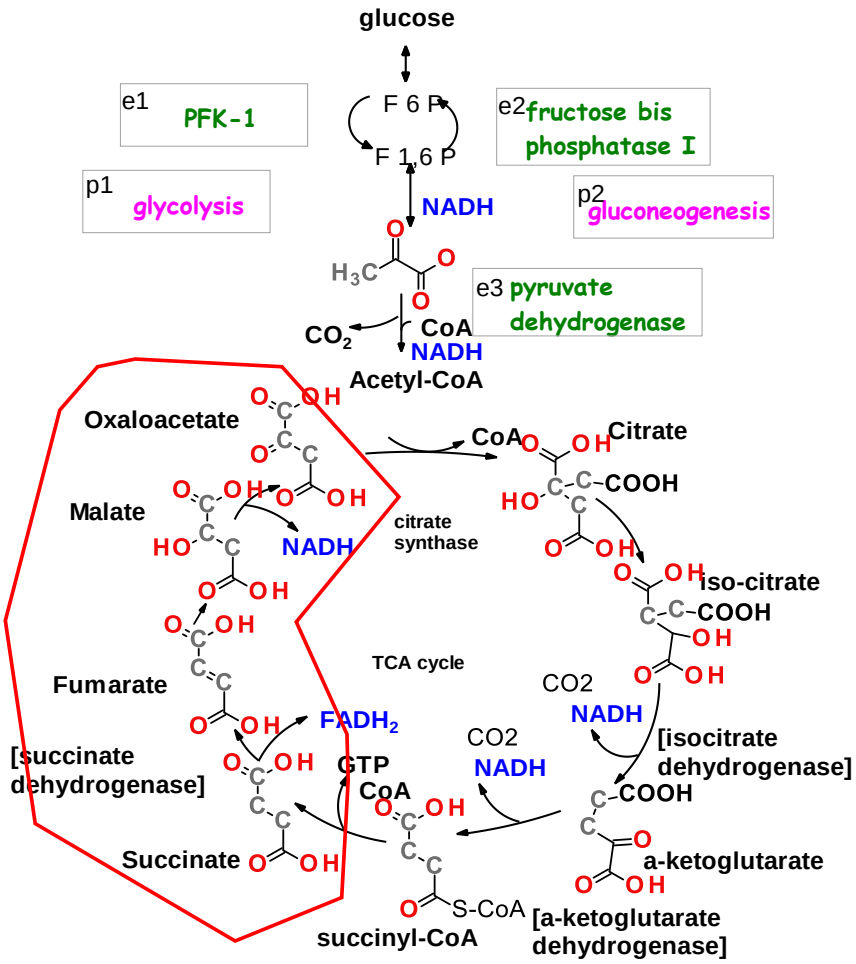
i) Please add the following labels:

a) names of two pathways (p1-p2)

b) names of three missing enzymes (e1-e3)

c) Indicate where NADH and $FADH_2$ are produced.

ii) Circle the part of the TCA cycle that has reactions that are identical in chemistry to fatty acid β-oxidation.



Oxidation of an alkane to a ketone.

17. (2 pts) In what way is the processing of $FADH_2$ different from NADH in electron transport.

$FADH_2$ is oxidized in complex II instead of complex I and does not pump protons (the rest of the electron path is identical – Q->III->IV->O_2)

Bonus (2 pts): A genetically inherited disease is a glycogen storage disease where the amount of glycogen in liver cells is much higher than normal individuals. These individuals make the same amount of glycogen synthase as normal individuals. What is the genetic defect/mutation?

Glycogen synthase cannot be shut off by phosphorylation, so perhaps the phosphorylation site (Ser, Thr, Tyr) has been mutated so that it can no longer accept a phosphate.

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