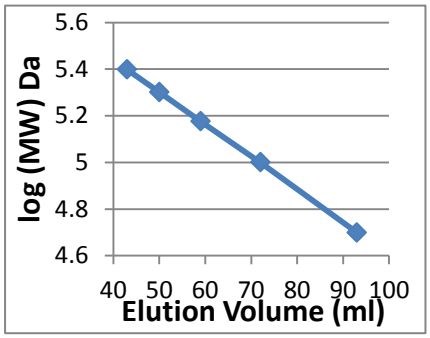
Instructions. This exam contains 17 questions and 100 points on 6 pages. Allot 1 min/2 points. On questions with choices, all of your answers will be graded and you will receive the grade for the best attempt.

1. (2 pts, Fill in the blanks). In structure determination by X-ray diffraction, the ___electrons____ in the atoms scatter the X-rays and the ___interference_ between the scattered X-rays allows the determination of the structure.

2. (6 pts) Determine the quaternary structure of a protein based on the data below. *Briefly justify your answer.*

a) Gel filtration chromatography. Elution volume=50 ml. The calibration curve for the column is given on the right. Some useful log values: *log*50,000=4.69, *log*100,000=5, *log*150,000=5.17, *log*200,000=5.3

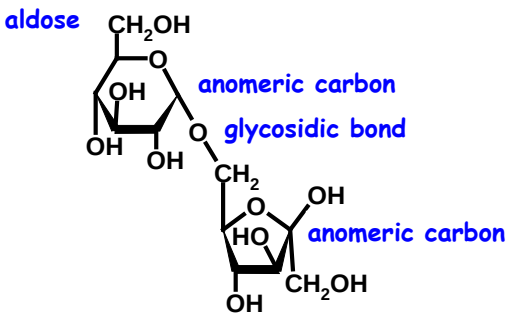
b) SDS-PAGE, two bands of equal intensity, one at 40,000 Da and the other at 60,000 Da.



The protein has the following quaternary structure – $\alpha_2\beta_2$ (3 pts)

Justification.
Since the bands on the SDS-page are of equal intensity the quaternary structure is $\alpha_n\beta_n$.
The native molecule weight is 200,000. If n=2, then the predicted molecular weight equals that obtained by gel filtration. (3 pts)

3. (3 pts) A disaccharide is shown on the right. Label the i) anomeric carbons, ii) glycosidic bond, iii) the aldose.



4. (3 pts) Select the correct name for the disaccharide shown on the right. *Briefly justify your answer in case you need partial credit.*

a) α-glucopyranosyl (1-6) β-frucofuranose
b) β-frucofuranose (6-1) α-glucopyranosyl
c) α-glucopyranosyl (1-6) β-ribofuranose
d) α-glucofuranosyl (1-6) β-frucopyranose

5. (6 pts) Please do one of the following three choices:
Choice A: Compare and contrast the structure of glycogen to cellulose.
Choice B: Describe the major features of a bacterial cell wall
Choice C: Why can lysozyme digest bacterial cell walls, but not cellulose?

Choice A: Both contain glucose (2 pts). Glycogen has α(1-4) and α(1-6) linkages, cellulose has β(1-4) (4 pts)

Choice B: Polysaccharide part consists of linear chains of alternating NAM, NAG, linked by β(1-4) (3 pts)
These are crosslinked by a peptide between the NAM units (2 pts)
NAG and NAM are modified glucose (1 pt)

Choice C: Lysozyme can hydrolyse β(1-4) bonds (2 pts), which are found in both cellulose and bacterial cell walls. It specifically recognizes the N-acetyl group on carbon 2 NAM/NAG instead of the –OH on cellulose (4 pts).

6. (3 pt) Which is the storage form of glucose in animals (circle answer). Glycogen *Starch* *Triglycerides*

7. (8 pts) Margarine is made by catalytic hydrogenation of vegetable oils, (e.g. corn oil), i.e. hydrogens are added to double bonds. Explain how this process converts the liquid oil to solid margarine. Be sure to mention any important molecular interactions and thermodynamic forces that are related to this process.

The fatty acids in the triglycerides in corn oil contain cis double bonds (1 pt)
The cis double bonds cause a kink in the acyl chain, which disrupts **van der Waals** interactions, lowering Tm such that the oil is a liquid (4 pts)
The addition of hydrogens converts the unsaturated cis double bonds to saturated bonds (1 pt)
The saturated bonds allow the fatty acids to become linear and pack with each other, enhancing their van der Waals interactions (2 pts)

8. (6 pts) What energetic/thermodynamics features or forces are common to both protein folding and the spontaneous assembly of lipid bilayers? Your answer should refer to the general structure of phospholipids.

Both are driven by the hydrophobic effect (release of ordered water when non-polar groups are buried) 3 pts.
Phospholipids have a general structure of 2 fatty acids esterified to C1 and C2 of glycerol. A phosphate is attached to carbon 3. The two fatty acids are non-polar and will form the interior of the bilayer. The phosphate is polar and charged, and will be on the surface of the bilayer, exposed to water. (3 pts)

9. (5 pts) Please answer **one** of the following choices.

Choice A: Why do fatty acids form micelles, while phospholipids form bilayers.

Choice B: Define the critical micelle concentration and briefly discuss how it would be affected by the length of the fatty acid.

Choice C: What will happen to the size of a phospholipid vesicle, whose interior is 0.1M NaCl, when placed in a solution of 0.5 M NaCl? Be sure to discuss the molecular bases for the permeability of water and ions in your answer.

Choice D: What is the role of cholesterol in membranes?

Choice A: Fatty acids have a head group (the carboxylate) that is larger in size than the non-polar part. Thus they are cone shaped. When cones are packed together, they will form a sphere. In phospholipids the area of the head group is roughly equal to that of the two acyl chains, so they are cylindrical. When they pack they will form a planer bilayer.

Choice B: The critical micelle concentration (CMC) is the concentration of fatty acids where micelles just begin to form. As the fatty acid becomes longer the CMC will drop, because the solubility of the fatty acid in water is decreasing.

Choice C: Water can cross the bilayer, but ions cannot (3 pts). The concentration of water is higher on the inside than the outside, so the water will leave the vesicle and it will shrink (2 pts).

Choice D: It increases the fluidity of the membrane (4 pts) allowing biological processes to occur, such as electron transport (1 pt).

10. (6 pts) Please answer **one** of the following choices.

Choice A: Membrane proteins are typically all α-helical or β-barrel. What is the thermodynamic basis for this property?

Choice B: A short peptide composed entirely of alanine residues (sidechain $-CH_3$) will not readily insert into a bilayer, even though the sidechain of alanine is clearly non-polar. Why?

Choice C: Briefly describe the molecular basis for the ion selectivity of the potassium channel, i.e. why is only potassium allowed through the channel, while other ions, like Na^+, cannot.

Choice A: The mainchain Hbond donors and acceptors will be forming H-bonds with water in the unfolded protein. When the protein folds those H-bonds are broken at a cost of +20 kJ/mol, which is energetically unfavorable. Since there are no H-bond donors and acceptors in the membrane, the protein has to form secondary structures that can reform all of these hydrogen bonds. The α-helical or β-barrel are two which can.

Choice B: The overall standard energy for partitioning of the peptide into the bilayer is composed of a contribution from the mainchain and sidechain atoms. The mainchain atoms are polar and it is unfavorable to insert them into the bilayer (by +1 kcal/mol, number not required). The alanine sidechain is sufficiently small that it cannot overcome this unfavorable energy change, it is necessary to have a larger non-polar side chain, such as valine.

Choice C: The ion must be dehydrated as it passes through the channel. This requires energy due to the favorable interaction of water with the charged ion. In the K channel there are a group of mainchain C=O groups where the oxygen interacts with the K+, replacing the favorable interaction with water with an interaction with the protein. Na+, which is smaller, cannot contact the C=O groups as well, so not enough energy is regained.

11. (6 pts) Please answer **TWO** of the following choices. For each choice state the name and location of all metabolic pathways the carbons flow through, and provide the names of key molecules, such as the input to the pathway, output from the pathway, and any intermediate molecules between the pathways. Feel free to draw a well labeled diagram for your answer.

Choice A: Outline the flow of carbon atoms from carbohydrates (.e.g. glucose) in metabolism.

Choice B: Outline the flow of carbon atoms from triglycerides in metabolism.

Choice C: Outline the flow of carbon atoms from amino acids, such as alanine, glutamic acid, aspartic acid, in metabolism.

Glucose → glycolysis (cytosol) → pyruvate (cytosol) → pyruvate (mito matrix) → Acetyl CoA →TCA (mito matrix) → CO_2

Triglycerides → fatty acid→fatty acid activation (cytosol) → acyl-CoA (cytosol) →acyl-CoA (mito matrix) → fatty acid oxidation (mito matrix) → acetyl CoA → TCA (mito matrix) → CO_2

Amino Acids → TCA (mito matrix) → CO_2

12. (8 pts) Overview of energy production and flow (please read Q13 before proceeding).

i) What is the general nature of reactions in metabolism that release energy? Give one example from **any** pathway [Hint: structures on the formula page may be useful.] (~~4 pts~~) (5 pts)

Oxidations, catalyzed by dehydrogenases (3 1/2 pts) Possible examples (1 1/2 pt) include:
a) glyceraldehyde-3-P dehydrogenase → 1,3 bis phosphoglycerate (glycolysis)
b) pyruvate → Acetyl CoA (entry to the TCA cycle)
c) Alkane → alkene (TCA cycle, fatty acid oxidation)
d)alcohol → ketone (TCA cycle, fatty acid oxidation)

ii) How is this energy captured in biological systems and not released as heat? (1 pt)

The energy released by the oxidation is captured by reducing NAD+ to NADH or FAD to FADH2

iii) What is the next step in the energy flow? (1pt, no need to give details, just state how the energy is stored, you can provide the details in Q13)

NADH/FADH2 enter the electron transport chain. When these are oxidized back to NAD+/FAD, the energy released is stored in a H+ gradient across the inner membrane.

iv) What is the final step in the energy flow? (1pt, again, no need to give details, just state what the step is. You can provide more details in Q13).

The hydrogen ions flow from high concentration to low, through ATP synthase. The energy released is stored as ATP.

13. (6 pts) Please do one of the following two choices.

Choice A: What is the role of complex II (succinate dehydrogenase) in the electron transport process? What is the path of electrons from complex II to the final electron acceptor?

Choice B: How does ATP synthase generate ATP from ADP and P_i?

Choice A: Complex II is part of the electron transport chain as well as the TCA cycle. Its role is to oxidize succinate producing FADH2, which is tightly bound by the enzyme. The electrons from complex II are transferred to coenzyme Q, which carries them to complex III. The electrons are transferred one electron at a time from III to IV by cytochrome C. In complex IV the electrons (4 e) are used to reduce O2 to H2O.

Choice B: **Choice B:**

- The enzyme that synthesizes ATP (ATP synthase, ATPase) resides in the inner mitochondrial membrane and matrix.
- The part that is in the matrix contains a gamma subunit, 3 alpha, and 3 beta. (2 pts for general description of enzyme)
- The conformation of the beta subunits depends on their interaction with the gamma, and this interaction changes every time 3 H+ pass through the enzyme. This is due to rotation of the gamma subunit, 120 deg/3 H+. (3 pts for stating effect of gamma on conformation of beta subunits)
- The initial conformation has low affinity for ADP and ATP. Thus ATP formed in the previous cycle would be released. The second conformation has high affinity for ADP and Pi, so they bind. The third conformation is such that ATP is lower energy than ADP + Pi, so the bound ADP + Pi is spontaneously converted to ATP. (1 pt)

14. (6 pts) Please do one of the following choices:

Choice A: Compare and contrast a product inhibitor to a feedback inhibitor (5 pts). Provide **one** example of **either** (1 pt).

Choice B: What are the covalent changes catalyzed by kinases and phosphatases? You may answer this question by giving the reaction scheme of one phosphatase and one kinase.

Choice A:

Both are used to regulate pathways (1 pt)

A product inhibitor inhibits the enzyme that generated it (2 pts). Examples acetylCoA inhibits pyruvate dehydrogenase, citrate inhibits citrate synthase.

A feedback inhibitor inhibits an enzyme earlier in the pathway (2 pts). Examples include succinyl-Coa inhibits citrate synthase. Citrate inhibits PFK in glycolysis.(1 pt for an example of either a product or feedback.)

Choice B:

A kinase transfers a phosphate from ATP to an –OH group. Examples include hexose kinase, PFK.

A phosphatase hydrolyses a phosphate using water. Examples include fructose bisphosphatase.

15. (10 pts) A reaction in a pathway converts A to B. The standard energy difference, ΔG^{o}, between A and B is large and positive, e.g. +15 kJ/mol.

i) Is this step likely to be spontaneous, based on the ΔG^{o}? Briefly justify your answer (4 pts)

It is unlikely to be spontaneous because the Gibbs energy is probably positive since $\Delta G = \Delta G^{o} + RT\ln[B]/[A]$

ii) How could you make this step spontaneous? Support your answer with an example from a metabolic pathway discussed in class (6 pts).

a) Direct coupling. In this case ATP would be used to phosphorylate A, generating B. The negative standard energy of ATP hydrolysis of -30 kJ/mol would make the standard energy -15 kJ/mol, which would likely make the reaction spontaneous. (5 pts) Examples: hexose kinase, PFK (1 pts).

b) Indirect coupling. In this case a reaction further down the pathways is energetically favorable such that it maintains the concentration of B below its equilibrium, this makes the ln[B]/[A] term negative, which would make the overall Gibbs energy negative. (5 pts) Examples, aldolase in glycolysis, malate dehydrogenase in the TCA cycle (last enzyme in cycle) (1 pt).

16. (6 pts) Please do one of the following choices:

Choice A: How are metabolic pathways sensitive (i.e. regulated by) the energy levels in the cell? Give one example, including the regulated step and how it is regulated.

Choice B: Under what conditions is ethanol produced from glucose? Approximately what percentage of the original energy in glucose is still contained in ethanol? 80% or 20%

Choice C: Why should athletes minimize their intake of fats as their primary energy source?

Choice A: When the cell has high energy reserves (high ATP and NADH) it shuts down glycolysis and the TCA cycle because these pathways generate energy and none is needed. These pathways are turned on when the cell needs energy, as indicated by high AMP and ADP levels. Example, PFK in glycolysis is activated by AMP and ADP but inhibited by ATP. Pyruvate dehydrogenase and citrate synthase are inhibited by ATP and NADH.

Choice B: Under conditions of low oxygen. Most of the energy in the original glucose is maintained in ethanol (80%)

Choice C: They will have trouble maintaining high levels of glycogen which would be used for rapid delivery of glucose while the athlete was performing. This is because carbon in fat cannot be converted to glucose because the step from Pyr to acetylCoA is one way in humans.

17. (10 pts) Select **one** of the following four choices and briefly describe how the pathway you picked (glycogen or glucose synthesis/degradation) is regulated by the blood glucose level (low or high) you picked. Be sure to mention any hormones that may be involved and the role of the hormone in the process. Also be sure to state the names of the enzymes that are regulated and how they are regulated. Finally, indicate that the regulation you described makes sense with respect to the needs of the cell or organism.

Choice A: Low blood glucose & glycogen syn/deg

a. Glucagon released by pancreas
b. Binds to its receptor and causes protein phosphorylation
c. Glycogen phosphorylase is active, releasing glucose from glycogen
d. The glucose can be used to increase the amount of glucose in the blood.

Choice B: Low blood glucose & glucose syn/deg

a. Glucagon released by pancreas
b. Binds to its receptor and causes protein phosphorylation
c. F26P levels drop due to activation of enzyme that degrades F26P
d. Gluconeogenesis is no longer inhibited, glucose is synthesized from pyruvate.
e. The glucose can be used to increase the amount of glucose in the blood.

Choice C: High blood glucose & glycogen syn/deg

a. Insulin released by pancreas
b. Binds to its receptor and causes protein dephosphorylation
c. Glycogen synthase is active, adding glucose to glycogen
d. The excess glucose in the blood is stored in glycogen.

Choice D: High blood glucose & glucose syn/deg

a. Insulin released by pancreas
b. Binds to its receptor and causes protein dephosphorylation
c. F26P levels rise due to activation of enzyme that makes F26P
d. Glycolysis is activated.
e. The liver cell can use the excess glucose to satisfy its energy needs.

Bonus (2 pts each):

A. Why is it better to put maple syrup on your pancakes instead of high fructose corn syrup?

Maple syrup is largely glucose whose metabolism is correctly regulated by PFK. Fructose enters glycolysis below PFK and is not a well regulated.

B. What is the purpose of generating thioesters in oxidations?

Storage of energy after oxidation. This energy can be used to form a carbon-carbon bond (citrate synthase) or generate GTP (Succinyl CoA synthetase)