

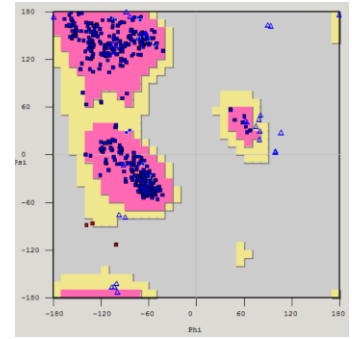
Instructions: this exam consists of 14 questions on 6 pages, for a total of 100 points. On questions with choices, all of your attempts will be graded and you will be awarded the highest grade.

1. (4 pts) X-ray Diffraction: Please do **one** of the following choices:

Choice A: What is the key principal that allows structures to be determined by the scattering (diffraction) of X-rays.

There is constructive interference between the X-rays scattered from atoms. Since the amount of interference depends on the relative position of the atoms, it is possible to use the scattering data to get the atomic positions.

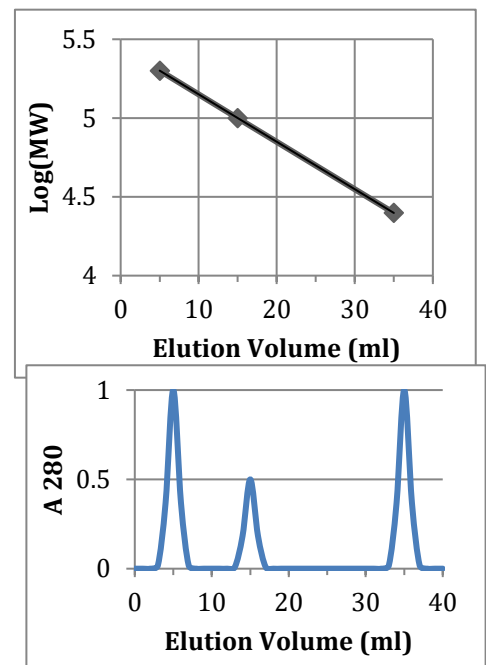
Choice B: The Ramachandran plot for a recently determined structure is shown on the right. Is the structure likely correct? Briefly justify your answer.



The structure is likely correct because the phi and psi angles of most of the residues fall in regions of low energy.

2. (8 pts) What is the quaternary structure of this protein, based on the following data? *Briefly justify your answer.*

- SDS-PAGE, **without** beta-mercaptoethanol (BME) gives two bands: 20 kDa, 30 kDa.
- SDS-PAGE, **with** beta-mercaptoethanol (BME) also gives two bands, 15 kDa, 20 kDa (15 kDa band is twice as intense).
- The elution profile and calibration plot for size exclusion chromatography is shown on the right. The size standards were 200 kDa ($\log 200,000=5.3$) and 25 kDa ($\log 25,000=4.4$). The unknown protein elutes at 15 ml.



- Comparing the SDS-PAGE with and without BME indicates that there are three peptides, one 20 kDa (β) and two 15 kDa (α). So, the minimum quaternary structure is $(\alpha_2\beta)$, with a minimum molecular weight of 50 kDa. The two α chains are linked with a disulfide bond.
- The native protein elutes at 15 ml, which on the log plot gives a y-intercept of 5, or an overall molecular weight of $10^5 = 100,000$.
- The quaternary structure that is consistent with all of these data is $(\alpha_2\beta)_2$, with a disulfide bond between the two α -chains.

3. (6 pts) for the dissacharide is shown on the right.

- Circle monosaccharide(s) that are ketoses.
- Indicate an anomeric carbon by circling it.
- Do one of the following choices

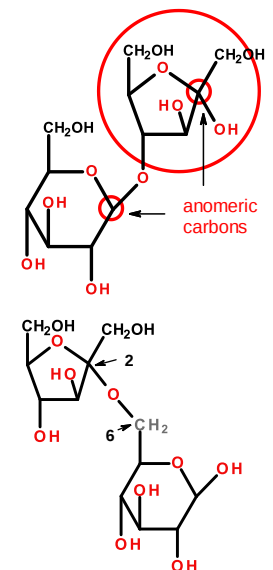
Choice A: Give the name of the sugar that is drawn.

Choice B: Draw the following sugar: α -fructofuranose (2-6) β -glucopyranose

- The right sugar should be circled; it is fructose, a 6 carbon ketose.
- Carbon 1 of the glucose (left sugar) or carbon 2 of the right sugar.
-

Choice A: The correct name is: β -glucopyranosyl-(1-4) α -fructofuranose:

Choice B: As drawn:



4. (6 pts) Please do **one** of the following choices. In either case you should indicate the monosaccharide component(s) and how they are linked together.

Choice A: What is the difference between lactose and sucrose?

Choice B: What is the difference between cellulose and glycogen?

Choice C: Briefly describe the structural features of the bacterial cell wall that result in its high mechanical strength.

Choice A:

- Lactose is a disaccharide of galactose and glucose, linked with a β (1-4) linkage.
- Sucrose is a disaccharide of α -glucose and β -fructose with the anomeric linked (1-2).

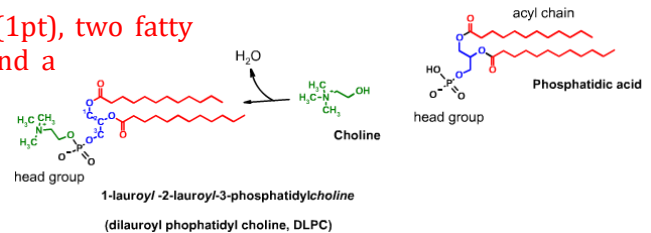
Choice B:

- Cellulose is a linear polymer of glucose, linked via β (1-4) linkages.
- Glycogen is a polymer of glucose with linear α (1-4) linked chains and α (1-6) branches.

Choice C: The linear polysaccharides of alternating NAM and NAG monosaccharides are covalently crosslinked with polypeptide chains. The polypeptide chains are linked to the NAM units and the protein chain off of each NAM is linked by a poly (5) glycine linker.

5. (6 pts) Draw **and** name a phospholipid of your choice.

- A correctly drawn phospholipid will have a glycerol core (1pt), two fatty acids in ester linkages to C1 and C2 of glycerol (1 pt) and a phosphate group on C3. (1pt) There can either be no additional groups or a choline or serine (linked through the alcohol) on the phosphate.(1pt)
- Naming: 1-fatty acid name, 2-fatty acid name, 3-phosphatidic acid (or phosphatidylcholine or phosphatidylserine.) (2pt)
- Naming the saturated fatty acid chains (based on the number of carbons in the fatty acid)- 12 lauroyl, 14-myristoyl, 16 palmitoyl, 18 stearoyl, 20 arachidonoyl, or unsaturated 18 with cis double bond at C9-10 oleoyl.



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

Choice A: Considering the structure of phospholipids, describe the thermodynamic/energetic features that explain why they form a bilayer in aqueous solutions.

Choice B: Explain how the critical micelle concentration changes when fatty acid tail length increases; will there be more or less free lipid in solution?

a. bilayers self-assemble in aqueous solution due to interactions of the polar head groups with the solution and the tails on the inside of the bilayer due to a strong hydrophobic effect. Van der Waals interactions also stabilize the bilayer.

b. the CMC will be lower the longer the fatty acid tail, meaning there will be less free lipids in solution as the tail length increase.

7. (4pts) In order to maintain membrane fluidity, the composition of fatty acids within phospholipids can be varied. Briefly describe the change that would occur to the length and saturation of the fatty acid tails as a cell is shifted to a lower temperature.

The newly made phospholipids would have more unsaturated fatty acids with shorter tails to help maintain a more fluid membrane at the lower temperatures.

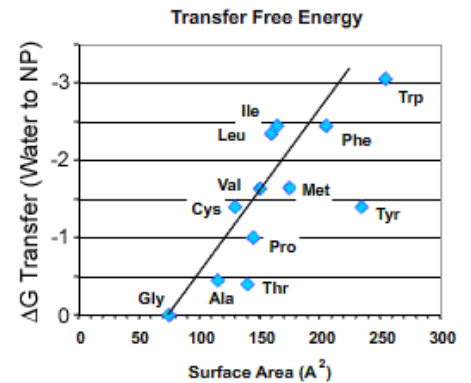
8. (10 pts) Please do **one** of the following choices.

Choice A: A peptide is made up of 10 tyrosine residues. Calculate the **partition coefficient** (equilibrium constant) for the peptide inserting into a membrane at 300K.

(Values for the diagram on the right are in kcal/mol. You should assume the transfer of the mainchain atoms is +1 kcal/mol and that $RT=600$ cal/mol at 300K.)

Choice B: Explain the structural features of integral membrane proteins; your answer should discuss both the secondary structure of the mainchain and the nature of the sidechain groups and the energetic features that are basis for these properties.

Choice C: Describe the overall structure of the potassium channel and explain its selectivity for K^+ ions.



a.

$$\Delta G = \Delta G_{\text{main}} + \Delta G_{\text{side}}$$

$$\Delta G = (1 \text{ kcal/mol}) + (-1.4 \text{ kcal/mol}) = -0.4 \text{ kcal/mol for one Tyrosine residue. Total would be -4 kcal/mol}$$

$$K_{\text{EQ}} = K_p = e^{-\Delta G/RT} = e^{(4000/600)} = 785$$

b. The main chain would be present in either an α -helix or β -barrel, as these are the only two structures that will satisfy all hydrogen bonds in the main chain atoms since no water is available in the membrane. Sidechains on the exterior of the protein that interact with the fatty acids would be non-polar, while either polar or non-polar residues would be present in the interior, depending on protein function.

c. The K^+ channel has a selectivity filter composed of a series of C=O groups that form a channel through which ions can pass. The K^+ ion must be desolvated to cross the channel. The unfavorable loss of water is countered by the favorable electrostatic interaction of the K^+ ion with C=O groups in the selectivity filter. The size of the filter only allows the correct interactions with K^+ .

9. (2 pts) A kinase uses ATP to add a phosphate group to its substrate.

Points on Page: _____

10. (10 pts) Answer **one** of the following questions:

Choice A: The conversion of glucose to glucose-6-phosphate by the addition of inorganic phosphate is an energetically unfavorable reaction. Describe how the cell overcomes this problem.

Choice B: Explain how indirect coupling of a reaction can allow an energetically unfavorable reaction to occur. Include an example from any pathway.

Choice C: Explain how glycolysis and gluconeogenesis can utilize many of the same enzymes in the breakdown and synthesis of glucose and both pathways can have a negative ΔG .

a. The cell can use direct coupling where a single enzyme transfers the phosphate group directly from ATP to glucose, thus coupling the energetically unfavorable reaction to a favorable one.

b. Indirect coupling is achieved by lowering the concentration of the product below its equilibrium concentration, thus favoring the forward reaction. This occurs when a reaction later in the pathway has a large negative ΔG . For example, by keeping the concentration of phosphoenolpyruvate low (due to its favorable conversion to pyruvate) the preceding reactions that convert fructose 1-6P through to PEP all proceed spontaneously. In fatty acid oxidation the production of acyl CoA is indirectly coupled to the energetically favorable hydrolysis of pyrophosphate.

c. Steps in each pathway that have large negative ΔG are performed by different enzymes while those with a $\Delta G \approx 0$ are reversible and kept favorable in each pathway by indirect coupling to the energetically favorable reactions that were performed by different enzymes (and often regulated).

11. (12 pts) Please answer **one** of the following choices on pathway regulation:

Choice A: Glycolysis, gluconeogenesis and the TCA cycle are each regulated by energy sensing. Choose one of these pathways and describe the step(s) where regulation occurs, including the molecules that enhance and/or inactivate enzyme activity and how that regulation makes sense for the cell.

Choice B: Insulin, epinephrine and glucagon each exert hormonal control over liver functions that can lead to the storage or release of glucose in glycogen. Pick one of the three hormones and explain their effect on protein phosphorylation and glycogen metabolism.

Choice C: Insulin, epinephrine and glucagon each exert hormonal control over liver functions that can lead to the metabolism or synthesis of glucose. Pick one of the three hormones and explain their effect on glucose metabolism (glycolysis and gluconeogenesis).

A. In glycolysis, the enzyme phosphofructokinase-1 (PFK-1) is positively regulated by AMP and ADP, thus increasing the rate of glycolysis and the production of ATP when energy levels are low and negatively regulated by ATP, thus turning off glycolysis when energy levels are high.

In gluconeogenesis, the enzyme fructose 1-6 bisphosphatase (FBP) is negatively regulated by AMP, thus decreasing the rate of gluconeogenesis and the production of glucose when energy levels are low.

In the TCA cycle, the enzymes pyruvate dehydrogenase and citrate synthase are negatively regulated by ATP and NADH, thus slowing the TCA cycle when energy levels are high.

B Insulin results in the activation of enzymes that are protein phosphatases, resulting in a lower level of phosphorylation of enzymes involved in metabolic pathways. As a result, glycogen synthase is activated while glycogen phosphorylase is turned off and glucose can be stored as glycogen.

Glucagon and epinephrine result in the activation of enzymes that are protein kinases, resulting in a higher level of phosphorylation of enzymes involved in metabolic pathways. As a result glycogen synthase is inactivated while glycogen phosphorylase is turned on and glycogen can be broken down to glucose to be released in the blood.

C. Insulin results in the activation of enzymes that are protein phosphatases, resulting in a lower level of phosphorylation of enzymes involved in metabolic pathways leading to the activation of phosphofructokinase-2 (PFK-2) and inactivation of fructose 2,6 biphosphatase. This increases the levels of Fructose 2,6 phosphate which activates phosphofructokinase-1 and turning on glycolysis and inactivating fructose 1, 6 phosphatase and turning off gluconeogenesis.

Signaling with glucagon and epinephrine result in the activation of enzymes that are protein kinases. This leads to inactivation of phosphofructokinase2 and activation of fructose 2,6 phosphatase resulting in decreased levels of Fructose 2,6 phosphate (F26P). Low F26P inactivates phosphofructokinase 1 and turns off glycolysis while activating fructose 1, 6 phosphatase and turning on gluconeogenesis.

12. (8 pts) A cell uses a proton gradient to produce ATP, requiring the transit of 3 H⁺ for each ATP synthesized. Assuming the pH in the inner membrane space is 6.5 and inside the matrix is 7.5, what is the minimum voltage difference required to provide enough energy to synthesize one ATP? T=300K.

$$\Delta G_{\text{TOTAL}} = \Delta G_{\text{CONC}} + \Delta G_{\text{ELEC}}$$

$$= RT \ln ([H^+]_{\text{IN}}/[H^+]_{\text{OUT}}) + ZF\Delta\Psi$$

If three protons must cross for each ATP and each ATP requires 30kJ/mol, then a minimum of 10kJ per proton is needed.

$$\Delta G = (8.31)(300) \ln(0.1) + (+1)(96,000 \text{ coulombs})(\Delta\Psi) = -10\text{kJ}$$

$$(-5740 \text{ J/mol} + 10000 \text{ J/mol})/96000 = \Delta\Psi = -0.045 \text{ V}$$

13. (10 pts) Answer one of the following questions:

Choice A: Describe how the electron transport chain is utilized to produce a proton gradient. Include a description of the flow of electrons through the transport chain.

Choice B: Describe how the proton gradient generated by the electron transport chain is utilized by ATP synthase to produce ATP. Be sure to describe the roles of the subunits of the F_o and F₁ complex in ATP synthesis.

a. In electron transport, the electrons flow from molecules with higher chemical potential to lower potential and ultimately reduce O₂ to produce H₂O. The flow of electrons is as follows:

NADH: Transfers two electrons to Complex I → CoQ → Complex III → Cytochrome C → Complex IV → O₂.

FADH₂: Transfers two electrons to Complex II → CoQ → Complex III → Cytochrome C → Complex IV → O₂.

As each of the complexes are reduced the captured energy allows the pumping of protons across the membrane in complexes I (4H⁺), III (4 H⁺), IV. (2H⁺) NADH = 10 H⁺ FADH₂ = 6 H⁺

b. The ATP synthase is present in the inner mitochondrial membrane is composed of two complexes, F_o which forms a transmembrane channel that allows the flow of protons and F₁, which has a γ subunit, 3 α subunits and 3 β subunits. The flow of protons through F_o causes the channel to rotate, which results in the rotation of the γ-subunit of the F₁ complex by 120° for every 3 H⁺. As γ rotates it can contact one of the three β subunits and influence their conformation. Each has 3 potential conformations, one that has a low affinity for either ADP and ATP, one that binds ADP and Pi with high affinity, and a third one that binds ATP with high affinity such that ATP is lower in energy than ADP and Pi, and thus the bound ADP and Pi are spontaneously converted to ATP.

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

Choice A: Briefly describe the steps involved in the metabolism of triglycerides **or** amino acids to produce ATP. Your answer should: i) including the names of major pathways, ii) their location, iii) key intermediates and their products. Feel free to draw a well-labeled diagram.

Choice B: When cellular levels of O_2 are limiting during strenuous exercise, glycolysis becomes the main source of energy. Describe what additional step(s) in either yeast or mammalian cells is (are) needed to allow continued utilization of glucose under the low O_2 conditions.

A. In the cytosol triglycerides are broken down to fatty acids which are activated to acyl-CoA and then these are transported into the mitochondrial matrix where β -oxidation produces acetyl CoA molecules that are oxidized to produce CO_2 in the TCA cycle.

Some amino acids are converted to keto acids by transaminases, the amino group is replaced by a C=O (keto) group. they can then enter the TCA cycle. Alanine enters as pyruvate, glutamic acid as keto-glutarate, and aspartic acid as oxaloacetate. This would all occur in the mitochondrial matrix. the carbons in the amino acids would be released as CO_2 .

B. Pyruvate, NADH and ATP are the products of glycolysis. To keep glycolysis running, there must be ample NAD^+ available for the glyceraldehyde-3-phosphate reaction. If O_2 is limiting, then NADH cannot be reduced using the electron transport chain and glycolysis cannot continue unless the NADH is oxidized another way.

In yeast pyruvate is converted to acetaldehyde, releasing CO_2 and the subsequent reduction of acetaldehyde to ethanol is coupled with the oxidation of NADH to NAD^+ .

In mammalian cells pyruvate is directly reduced to lactate and NADH is oxidized to NAD^+ , allowing glycolysis to continue.

You may do **two** of the following bonus questions (2 pts each).

Bonus (2 pts): Briefly explain how either penicillin or lysozyme kill bacteria.

- penicillin inhibits the formation of the peptide crosslinkage in the cell wall.
- lysozyme can hydrolyze the $\beta(1-4)$ linkage between the NAM and NAG units.

Bonus (2 pts): Why is it important for sprinters to “carbo-load” before a race ?

Sprinters need to produce energy very quickly, which can be done with glycolysis. Therefore they need higher glycogen stores.

Bonus (2 pts): Why is it difficult to convert the carbons in olive oil to glucose?

The triglyceride would be broken down to fatty acids, which are oxidized to acetyl-CoA. This cannot be converted to pyruvate in humans.