Biochemistry Spring 2015 Exam III Name:_____

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

1. **(4 pts)** You are interested in thymidylate kinase from E.coli. You know the amino acid sequence of the protein, from which you predict an isoelectric pH (pI, <q>=0) of 7.0. Please answer one of the following choices.

Choice A: Briefly describe how you would use this information to design one of the steps in the purification of the protein.

Choice B: Outline how you could determine the structure of thymidylate kinase using X-ray diffraction methods.

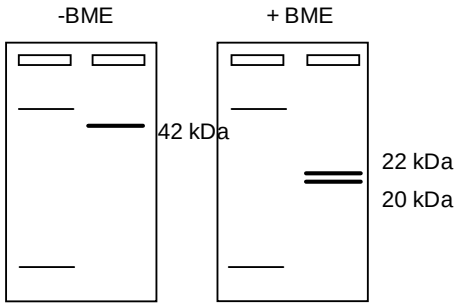
Choice A

- Since you can predict the charge as a function of pH you could use ion exchange. At pH < pI the protein will be positively charged and would stick to a cation exchange resin. Some impurities could be washed away, and then the protein eluted with salt.
- Since this is a kinase, it uses ATP as a substrate. You could make an affinity column with ATP, the protein will bind and you can wash away impurities. This would not be a single step purification because many other enzymes would also bind ATP.

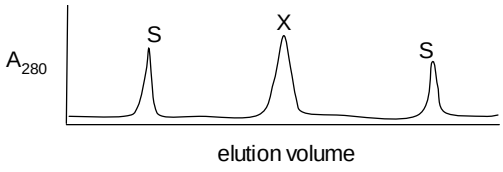
Choice B: Crystalize it, collect diffraction data, generate an electron density map, fit the atoms into the map.

2. **(6 pts)** After purifying the protein you wish to determine its quaternary structure. Please answer **one** of the following choices.

Choice A: You run SDS-PAGE gels without and with BME (β-mercaptoethanol). Images of the two gels are shown on the right. The left lane contains the standards and the right lane is the sample. The molecular weights of the proteins are indicated on the gel images. What is the minimum quaternary structure of the protein based on these data?



Choice B: You run a gel filtration (size exclusion) and the elution profile is shown on the right. The size of the protein (marked X) is 85 kDa. How would you have determined this from the data shown on the right?



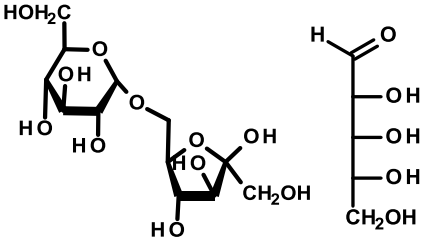
Choice A: There are two subunits, linked by a disulfide bond. The presence of the disulfide bond is indicated by a single band when BME is omitted. The subunits are 1:1 because the two bands in the right gel are the same intensity.

- **Minimum quaternary structure α-β.**

Choice B: The molecular weights of the standards are known, so you would generate a plot log MW versus elution volume. This plot can be used to convert the known elution volume of X to the log of its molecular weight.

3. **(6 pts)** Please do **one** of the following choices.

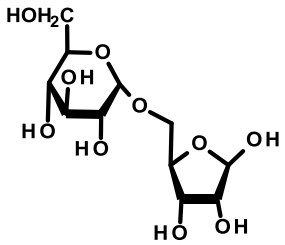
Choice A: The following is a disaccharide of glucose (top) and fructose (bottom). Please name this sugar using standard nomenclature.



Choice B: Draw the following disaccharide: α-glucopyranosyl – (1-5) β-ribofuranose. Ribose is a C5 aldose and its linear structure is shown on the right.

Choice A: α-glucopyranosyl (1-6) β-fructofuranose

Choice B: Shown on the right.



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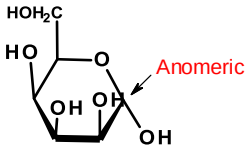
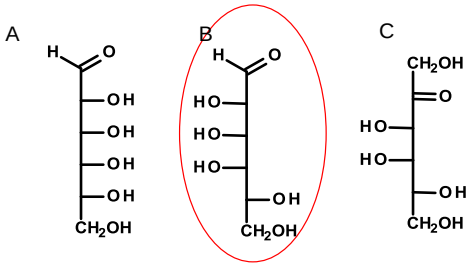
4. **(2 pts)** Circle the correct linear form of the cyclic sugar and indicate the anomeric carbon.





OH groups that are up on the ring are to the left on the linear form.
Although the anomeric carbon is created when the sugar becomes cyclic, it is acceptable label it on the linear form.

5. **(6 pts)** Please do one of the following choices:

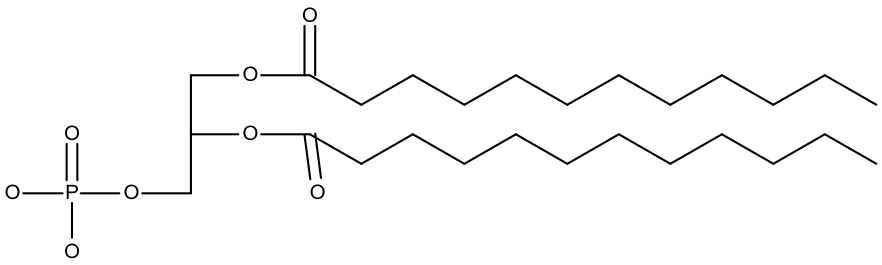
Choice A: Compare cellulose versus glycogen (answer all parts).
i) How does the structure of cellulose differ from glycogen?
ii) In what way are these polysaccharides similar?
iii) Which is used for energy storage in mammals?

Choice B: Compare and contrast bacterial cell walls and cellulose.

***Choice A*:**
Both are polymers of glucose, cellulose has β (1-4) linkages, glycogen has α(1-4) and α(1-6) branches.
Glycogen is used for energy storage.

***Choice B*:**
Both are linear polymers with either glucose (cellulose) or modified glucose (NAM, NAG) linked by β(1-4). In case of bacterial cell walls the NAM units are crosslinked by peptide chains.

6. **(6 pts)** Draw ***and*** name any phospholipid that you like. The following information may be useful: lauric C_{12}, myristic C_{14}, palmitic C_{16}.



Dilauryl phosphatic acid would look like.
5 pts for correct structure
1 pt for correct name

7. **(6 pts)** Please do one of the following choices.

Choice A: What is the critical micelle concentration and what is its relationship to detergents?
Choice B: What is the principal structural difference between a phospholipid and a fatty acid and how does this difference affect the structures these compounds form in water?.
Choice C: Corn oil is a triglyceride that has unsaturated double bonds as part of its fatty acid component. Why is it a liquid at room temperature?

***Choice A*:** CMC is the highest concentration which monomeric fatty acids can exist. If the concentration is above the CMC micelles will form. The micelles will have better detergent activity.

***Choice B*:** Fatty acids have one non-polar tail and form micelles due to the larger polar headgroup. Phospholipids have two polar tails and since the headgroup is the same area as the tails, they form bilayers.

***Choice C*:** The unsaturated double bonds are cis, and cause a bend in the chain – disrupting van der Waals activity and lowering the melting temperature.

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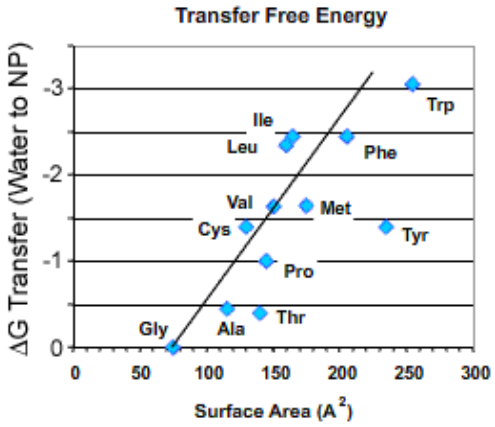
8. **(6 pts)** A 20 residue protein consists entirely of alanine residues. Please do **one** of the following choices.

Choice A. When this protein is added to a solution of phospholipid vesicles very little of the protein enters the bilayer. Why?

Choice B. What is the most likely secondary structure(s) of any proteins that are inserted into the membrane? Why?



Choice A: Burying the protein mainchain atoms is unfavorable since they are polar. The nonpolar sidechain of Ala is too small to overcome this unfavorable energy.

Choice B: The only possible secondary structures are an α-helix or a β-barrel. This allows the reformation of H-bonds that were to water when the protein is in the membrane. This is essential since there are no H-bond donors or acceptors in the membrane.

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

Choice A. Briefly describe why the potassium channel is selective for K^+ ions.

Choice B. Why can the potassium channel be considered an enzyme?

Choice C: Why do cells or membrane vesicles swell when placed in hypotonic (lower salt concentration outside) solutions?

Choice A: The mainchain carbonyl groups in the selectivity channel interact strongly with the K^+ ion so that it can be desolvated. Smaller ions (Na) remain solvated, and larger ions (Rb) are just too big.

Choice B: Because it catalyzes a reaction, the movement of K across the membrane.

Choice C: The water concentration outside is higher than inside, so the water flows from high to low, i.e. outside to in.

10. **(6 pts)** Glycolysis converts glucose to pyruvate. Why is it necessary to perform some steps by a different mechanism in gluconeogenesis? (5 pts) Give an example of one of those steps (1 pt)

The steps are too high in energy drop to be reversed by indirect coupling. All other steps can be reversed due to indirect coupling (the last step in both pathways is very favorable). This also provides a means to independently regulate each pathway. The three steps are:

a) Glucose → Glucose-6-P
b) F6P → F16P
c) PEP→Pyruvate.

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11. **(2 pts)** What is the difference between a feedback inhibitor and a product inhibitor?

A product inhibitor inhibits the enzyme that generated it.

A feedback inhibitor inhibits a step earlier in the pathway.

12. **(8 pts)** Briefly describe the difference between direct coupling and indirect coupling and why coupling of one type or another is usually required in biochemical pathways. Provide **one** example of **either** type of coupling.

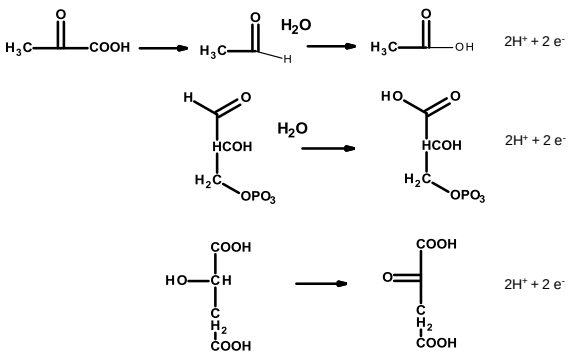
Direct coupling uses the energy of ATP hydrolysis (ATP →ADP + Pi) to make a reaction with an unfavorable standard energy (ΔG°) favorable. Both ATP and the other substrate bind to the same site. Examples:

- Hexose kinase (glucose→glucose-6P), phosphofructose kinase (F6P→F16P). Fatty acid activation (Fatty acid→acyl-CoA)

Indirect coupling works by keeping the concentration of products below their equilibrium level, making the RTln[B]/[A] term in the Gibbs energy negative.

- The last step in glycolysis (PEP→Pyruvate) or gluconeogenesis (G-6-P→G) do this to keep the pathways spontaneous.

13. **(7 pts)** For any **one** of the following reactions shown on the right:



- Pyruvate to Acetyl-CoA (the thiol ester has the same oxidation state as a carboxylic acid).

  The first step is loss of CO_2 to form the aldehyde. So the redox step is oxidation of the aldehyde to a carboxylic acid. To balance the reaction you have to add H_2O to the left, and $2H^+$ and 2 e^- to the right.

- Glyceraldehyde-3-Phosphate to 1,3-phosphoglycerate (the phosphate ester has the same oxidation state as a carboxylic acid).

  The redox step is oxidation of the aldehyde to a carboxylic acid. To balance the reaction you have to add H_2O to the left, and $2H^+$ and 2 e^- to the right.

- Malate to oxaloacetate

  This is oxidation of an alcohol to a ketone. Oxygens are already balanced, need to just add $2H^+$ and 2 e^- to the right.

i) Show, by balancing, that the reaction is a redox reaction (4 pts). Note: was originally 2 pts.

ii) What is the general name of the type of enzymes that catalyze this type of reaction (1 pt).

Dehydrogenases

iii) These reactions release energy, how is it captured for use by the cell and not lost as heat (2 pts)?

NADH is formed, which requires energy.

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14. **(9 pts)** Please do one of the following choices:

Choice A: Briefly describe how either NADH or $FADH_2$ are processed by electron transport. Be sure to discuss the following: i) the final electron acceptor, ii) how is the energy released by oxidations stored for ATP synthesis, iii) How would you calculate the energy available for ATP synthesis.

Choice B: Briefly explain how ATP is synthesized in the mitochondria. What is the energy source and briefly describe the mechanism by which ATP is formed from ADP and P_i. How would you calculate the energy available for ATP synthesis?

Choice A:

NADH – oxidized by complex I, electrons transferred to Q, delivered to complex III, shuttled to complex IV where they are used to reduce O_2 to water. The oxidations pump hydrogen ions across the inner mitochondrial membrane, storing the energy (~10 H^+ pumped)

$FADH_2$ – oxidized by complex II, electrons transferred to Q, delivered to complex III, shuttled to complex IV where they are used to reduce O_2 to water. The oxidations pump hydrogen ions across the inner mitochondrial membrane, storing the energy (~6 H^+ pumped).

The energy stored is $\Delta G = RT \ln [H^+]_{in}/[H^+]_{OUT} + ZF\Delta V$

Choice B:

The hydrogen ion gradient formed during electron transport is the source of the energy. The amount of energy is: $\Delta G = RT \ln [H^+]_{in}/[H^+]_{OUT} + ZF\Delta V$.

The enzyme consists of an Fo part (in the membrane) and an F1 part in the matrix. The F1 part synthesizes ATP and contains 3 β subunits and a gamma subunit.

The protons go through the Fo part

$3H^+$ cause a 120 degree rotation of the gamma subunit

Rotation of the gamma subunit changes the conformation of the β-subunit, such that a single β subunit cycles:

1. Low affinity (previously made ATP released)
2. High affinity for ADP and Pi (so they bind)
3. Energy of ATP lower than ADP + Pi, so ATP forms spontaneously.
4. Low affinity (ATP released)

Total of 9 H^+ required for one cycle = 3 ATP produced.

15. **(6 pts)** Please do one of the following choices.

Choice A: Describe how fatty acids are prepared (activated) for oxidation. Include a description of the chemical changes and the cellular location.

Choice B: Describe the process of fatty acid oxidation, including a statement of cellular location.

Choice C: The TCA cycle is typically thought of as a degradative pathway. Provide **one** example of how intermediates in the TCA cycle can be used in a biosynthetic pathway.

Choice D: Briefly explain why a person on a high fat diet will have low levels of stored glycogen, especially after strenuous exercise.

Choice A: In the cytosol fatty acids are converted to acyl-CoA, forming a thio-ester. This requires two high energy phosphate bonds (ATP→AMP).

Choice B: Occurs in the mitochondrial matrix. B-carbon is oxidized to a ketone by a three step process (alkane – alkene – alcohol – ketone). A CoA attacks the β-carbon releasing an acetyl-CoA, forming an acyl-CoA that is two carbons shorter.

Choice C: To intermediates in the TCA cycle, oxaloacete and ketoglutarate can be converted to amino acids (Aspartic acid, Glutamic acid). Pyruvate can be converted to Alanine.

Choice D: The carbon in fatty acids is converted to acetyl-CoA. In order to make glucose (and then glycogen) from this carbon it must be converted to pyruvate. Humans do not have an enzyme to do this.

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16. **(10 pts)** Please do **one** of the following choices.

Choice A: Briefly explain how glycogen metabolism **or** glycolysis/gluconeogenesis are controlled by hormones under conditions of **high** blood glucose.

Choice B: Briefly explain how glycogen metabolism **or** glycolysis/gluconeogenesis are controlled by hormones under conditions of **low** blood glucose.

Choice C: Briefly explain how glycolysis/gluconeogenesis **are** controlled by energy sensing in the cell.

Choice A-High blood glucose will cause the release of insulin from the pancreas
Binding of insulin to its receptor will cause dephosphorylation of enzymes.

- Glycogen metabolism:
  - Glycogen synthase is active when dephosphorylated – so glucose is stored in glycogen.
  - Glycogen phosphorylase, which releases glucose from glycogen, is inactive.
    It was necessary to discuss both enzymes for full credit.
- Glycolysis, gluconeogenesis:
  - F26P levels are high because the enzyme that makes F26P is active when dephosphorylated.
  - Glycolysis may be on (see below for energy sensing) since PFK is activated by F26P.
  - Gluconeogenesis is off since bisphosphatase is inhibited by F26P.

  It was necessary to discuss both enzymes for full credit.

Choice B -Low blood glucose will cause the release of glucagon from the pancreas
Binding of glucagon to its receptor will cause phosphorylation of enzymes via a G-protein coupled receptor

- Glycogen metabolism:
  - Glycogen synthase is inactive when phosphorylated.
  - Glycogen phosphorylase, which releases glucose from glycogen, is active when phosphorylated, so glucose would be released,
- Glycolysis, gluconeogenesis:
  - F26P levels are low because the enzyme that degrades F26P is active when phosphorylated.
  - Glycolysis is off since PFK is only active when F26P is present.
  - Gluconeogenesis may be (see below for energy sensing) on since bisphosphatase is no longer inhibited by F26P.

Choice C:

- If ATP levels are high glycolysis will be off because PFK is inhibited by ATP – there is no need for the cell to make it.
- Gluconeogenesis could be active (provided F26P levels are low due to low blood glucose), because the cell has sufficient resources to make glucose from pyruvate. Bisphosphatase in gluconeogenesis is inhibited by AMP, which would be low if ATP levels are high.
- If ATP levels are lower, AMP and ADP will be high. This will turn on glycolysis (PFK is activated by AMP and ADP), provided F26P levels are high, indicating high blood glucose. Bisphosphatase in gluconeogenesis is off, since it is inhibited by AMP.

17. **(5 pts)** The country of Brazil does not import any petroleum products. What fuel is used to power automobiles in that country and how is that fuel made?

Ethanol, from anaerobic metabolism of glucose by yeast (fermentation). Glucose from sugar cane is the source of the starting material.

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