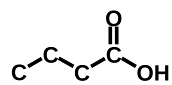
## This exam contains 9 pages. There are a total of 90 points. Allot 1 min/point.

**Part A**: (17 pts) Multiple Choice (11 x 1½ pts each = 15.5 pts) and Fill in the blanks (1.5 pts).

Please circle the best answer.

1. The sub-unit molecular weight as well as the number of sub-units in the quaternary structure can be determined by:
   a) SDS-PAGE electrophoresis. **(+1/2 pt)**
   b) Ion exchange chromatography
   c) Gel filtration chromatography. **(+1/2 pt)**
   **d) Combining information from a) and c)**

2. Which of the following fatty acids would have the lowest CMC (Critical Micelle Concentration)?
   a) $C_4$-COOH **(+1/2 pt)**
   b) $C_6$-COOH
   c) $C_8$-COOH
   **d) $C_{10}$-COOH [The CMC is the highest concentration of free fatty acid. The more non-polar the fatty acid, the lower concentration of individual fatty acids – they would prefer to enter the micelle.]**

**$C_4$-COOH =** 


3. Cholesterol is essential for normal membrane functions because it
   a) activates ATP synthase.
   b) spans the total thickness of the bilayer.
   **c) keeps membranes fluid.**
   d) carries electrons in electron transport.

4. A small 20 residue peptide will be largely found buried in a phospholipid bilayer membrane if:
   a) its sidechains are all protons (e.g. glycine). **(+1/2 pt)**
   b) its sidechains are all $CH_2$-OH groups (e.g. Serine).
   c) its sidechains are all $CH_3$ groups (e.g. Alanine). **(+1 pt) [Alanine is not sufficiently non-polar]**
   **d) its sidechains are all isopropyl groups (e.g. Valine).**

5. Energy for the synthesis of ATP (Chemical energy) during *oxidative* phosphorylation is obtained from:
   a) High energy phosphate compounds, such as phosphoenolpyruvate.
   b) A proton gradient across the cell membrane. **(+1/2 pt)**
   c) A proton gradient across the outer mitochondrial membrane. **(+1 pt)**
   **d) A proton gradient across the inner mitochondrial membrane.**

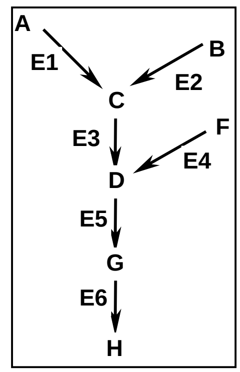
6. Opposing degradative and biosynthetic pathways
   a) use the same enzymes for many steps.
   b) use different enzymes for steps that require large energy changes.
   c) are coordinately regulated at steps that use different enzymes. **(+1 pt)**
   **d) all of the above.**

7. Which of the following compound is responsible for the coordinated regulation of glycolysis and gluconeogenesis by hormonal control?
   a) NADH
   b) acetyl-CoA
   **c) fructose 2,6 bis-phosphate**
   d) fructose 1,6 bis-phosphate

8. The TCA cycle is involved in:
   a) generation of energy from pyruvate **(+1/2)**
   b) synthesis of amino acids **(+1/2)**
   c) degradation of amino acids **(+1/2)**
   **d) all of the above**

9. Long-chain fatty acids are oxidized step-wise in ____ carbon units, producing ____.
   a) 1, formaldehyde
   b) 2, pyruvate.
   **c) 2, acetyl-CoA**
   d) 4, succinyl-CoA

10. During ATP synthesis, the three otherwise identical β subunits of the F1 complex
   a) have similar affinities for ADP and ATP.
   b) have different affinities for ADP but not for ATP.
   c) have different affinities for ATP but not for ADP.
   **d) have different affinities for ATP and for ADP.**




11. In the metabolic pathway shown to the right, enzyme _____ would likely be regulated and ______ would be an example of a feedback inhibitor.
   a) E1, C
   b) E1, H **(+1 pt)**
   c) E5, G **(+1 pt)**
   **d) E5, H**

Bonus Question (1 pt): Caffeine is a stimulant because:
   a) It accelerates the decay of cAMP.
   b) It accelerates the decay of ATP.
   **c) It slows the decay of cAMP.**
   d) It binds directly to excitory neurons.

**Fill in the blanks**: You need only fill in **one** sentence. (½ pt each word, 1.5 pts total.)

1. Anaerobic metabolism produces ___**lactate**____ in humans, _____**ethanol (alcohol)**_______ in yeast, and

   regenerates NAD+ for ______**glycolysis**_________ (name of metabolic pathway) in the absence of oxygen.

**OR**

2. The metabolic pathway ______**glycolysis**______ occurs in the cytosol, as does fatty acid

   _____**activation**____ (activation/oxidation) whereas ____**oxidation [or TCA cycle]** _____ occurs in the

   mitochondrial matrix.

**1.** (4 pts) *Briefly* describe how X-ray diffraction can be used to determine the structure of a protein.

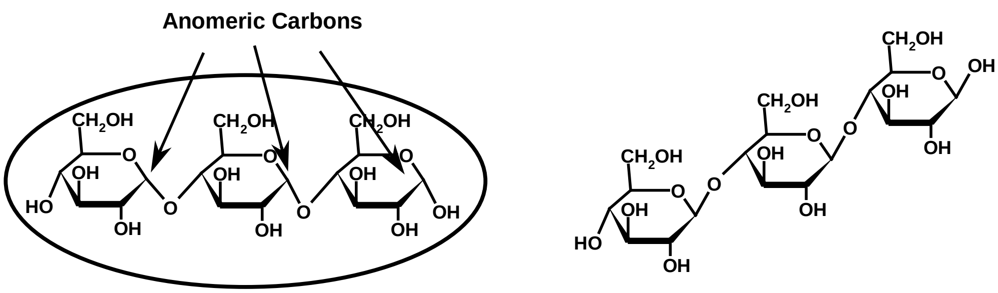
1. **X-rays are scattered by electrons on atoms.**
2. **Scattering depends on position of atoms.**
3. **Electron density map is calculated from scattering.**
4. **Atoms are fit into electron density map.**

**2.** (7 pts) Please select **one** of the following two choices.

**Choice A**:

i) Circle the trisaccharide that would be found in starch and glycogen. *Briefly* justify your answer.(3 pts)

**Starch and glycogen have α(1-4) linkages between the sugars. These are present in the circled molecule. The other trisaccharide has β(1-4) linkages – it is cellulose.**




ii) Indicate the location of an anomeric carbon.(2pts)

iii) What monosaccharide are these trisaccharides made of (2 pt)?

**Glucose**

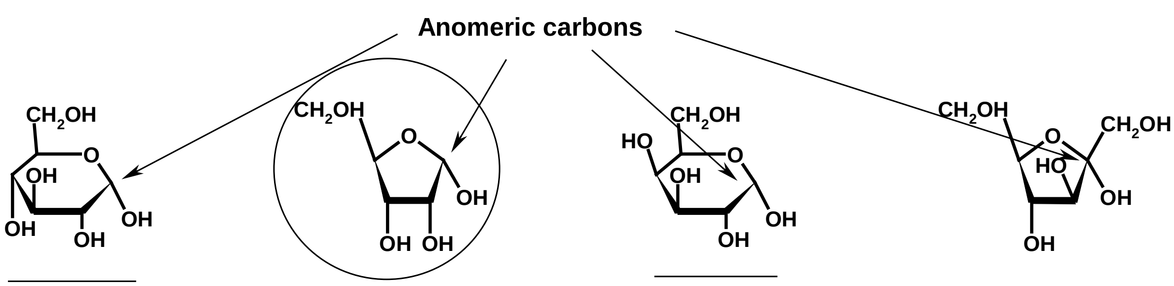
**Choice B:**

i) Circle the sugar that is an aldose found in nucleic acids.(2pts)

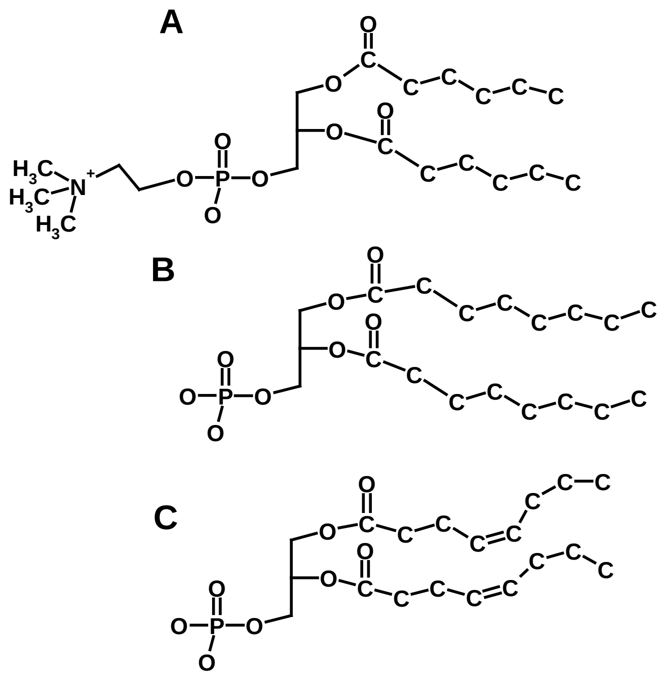
ii) Underline the two epimers (2 pts)

iii) Indicate the location of an anomeric carbon. What is its configuration in all four sugars? (3 pts)

**The –OH group on the anomeric carbon is pointing "down" – which is α.**



**3.** (12 pts) The drawing to the left shows three phospholipid molecules, labeled A, B, and C.




i) (1 pt) Add *any* head group to the top lipid and give its name in the space below (e.g. fill in the following blank):

dihexanoyl phosphatidyl **Choline**

ii) (3 pts) Briefly describe how would the structure of a triglyceride would differ from these lipids.

**The phosphate group would be replaced by another fatty acid.**

iii**)** (8 pts) Please do **one** of the following three choices. Please indicate your choice.

**Choice A:** Describe the role of the hydrophobic effect in the formation of micelles *or* lipid bilayers.

**In the bilayer the non-polar acyl chains are removed from water due to the hydrophobic effect.Free phospholipids will have water ordered around the non-polar acyl chain. When the bilayer forms, these water molecules will be released, increasing the entropy of the system, which is favorable.**

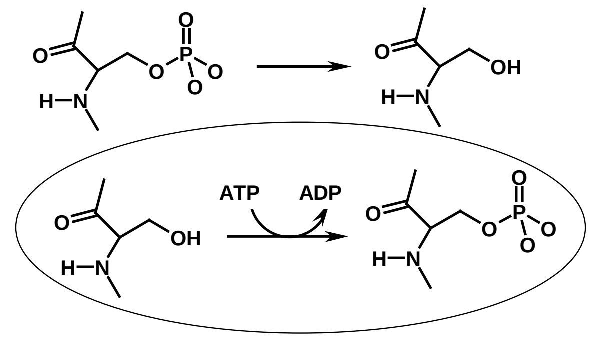
**Choice B:** Why are the secondary structures of all integral membrane proteins α-helical or β-barrel.

**There are no hydrogen bond donors or acceptors in the non-polar part of the bilayer. Therefore the protein must form all mainchain hydrogen bonds. This is only possible with an α-helix or a β-barrel. The energetic cost of breaking a H-bond to water and not reforming an H-bond when the protein is in the membrane is +20 kJ/mol.**

**Choice C:** The trend in melting temperature, $T_M$, for the above lipids is: C (lowest), A, and then B. Briefly explain this trend. Your answer should discuss the molecular forces/interactions that affect $T_M$.

**The melting temperature is determined by the van der Waals contacts. The longer the acyl chain the higher the melting temperature. Since B has longer acyl chains, its melting is higher than A. C is the lowest because the cis double bonds kink the chain, disrupting the van der Waals contacts.**

**4.** (8 pts) Please do **one** of the following two choices. Note that each choice has multiple questions. Answer all of the questions within a choice.




**Choice A:** Two reactions are shown to the right.

i) (2 pts) *Circle* the reaction catalyzed by a kinase.

ii) (2 pts) What is the general name of the other reaction?

**Phosphatase**

iii) (4 pts) *Briefly* discuss the role of reactions of this type in the *regulation* of enzymes. Give an example.

**The phosphorylation of Serine (shown) and Tyrosine residues (not shown) is a form of allosteric regulation, activating or inhibiting an enzyme. Examples are:**

- ***Glycogen synthase: Phosphorylation inactivates.***
- ***Glycogen phosphorylase: Phosphorylation activates.***

**Choice B:** Six reactions are shown to the right. Note: not all co-factors/co-substrates are shown.

i) (2 pt) *Circle* the one reaction that is **not** a redox reaction.

ii) (2 pt) What do the remaining redox reactions have in common?

**They are all oxidations.**

iii) (2 pts) What cofactors or co-substrates would be required for most of these reactions?

**$NAD^+$ (NADH), FAD ($FADH_2$)**

iv) (2 pts) For the last reaction (F) and **one other** redox reaction (draw a *box* around your selection), state which metabolic pathway (Glycolysis, TCA, Fatty acid oxidation, electron transport) reactions of these types can be found.

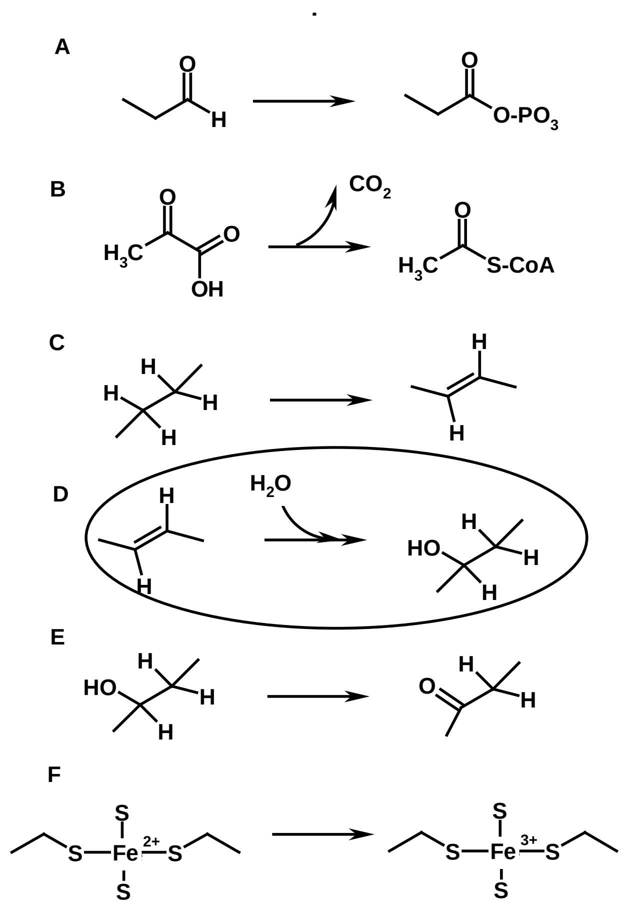
**A: glycolysis**

**B: TCA cycle**

**C: Fatty acid oxidation or TCA cycle**

**E: Fatty acid oxidation or TCA cycle**

**F: Electron transport.**



**5.** (12 pts). Please answer one of the following four choices. Please indicate your choice.

**Choice A:** The South Beach diet suggests that the dieter completely eliminate carbohydrates from their diet. Should athletes with high energy demands, such a sprinters, go on this diet? Why or why not?

**Sprinters need a fast source of energy. This can only be provided from glucose in glycolysis.**
**The glucose is released from glycogen.**
**Glycogen storage is elevated in high carbohydrate diets.**
**Fats and proteins cannot be efficiently converted to glucose because acetyl-CoA can't be converted to pyruvate in humans.**

**This diet is a bad idea.**

**Choice B:** List, in the correct order, *all* of the metabolic pathways that would be used in the generation of ATP from cream cheese (fats and proteins) and a bagel (starch).

**Starch → glucose → Glycolysis → TCA → electron transport → ATP synthesis.**

**Fats → Fatty acid oxidation → TCA → electron transport → ATP synthesis.**

**Proteins → amino acids → TCA → electron transport → ATP synthesis.**

**Choice C:** Discuss the electron transport process, beginning with NADH and ending with the production of $H_2O$. You should *not* discuss ATP synthesis. A *well labeled* diagram is an acceptable answer.

**Four complexes in inner mitochondrial membrane,**

**I: Oxidizes NADH to $NAD^+$, electrons go to CoQ.**
**II: Oxidizes $FADH_2$ to FAD, electrons go to CoQ.**
**III: Takes electrons from CoQ, passes them to cytochrome C**
**IV: Takes electrons from cytochrome C, passes them to $O_2$**

**Complexes I, III, IV pump protons, creating $H^+$ gradient used to synthesize ATP.**

**Choice D:** Discuss the mechanism of ATP synthesis in the mitochondria. Your answer should indicate the source of energy for ATP synthesis. You should *not* discuss electron transport. A *well labeled* diagram is an acceptable answer.

**ATPsynthase consists of two parts Fo – in the membrane, F1 – three α, three β subunits. Gamma subunit interacts with β subunits in an asymmetric way – therefore the affinity of each ADP/ATP to each β is different.**

**i) doesn't bind ADP/Pi or ATP**
**ii) binds ADP & Pi strongly**
**iii) binds ATP strongly.**

**Focusing on just <u>one</u> B-subunit. It is initially in state i. Proton passage through the Fo part causes rotation of gamma, converting the β-subunit to state ii. ADP + Pi bind. 2nd rotation causes state to go to iii, since ATP is more stable, it forms. 3rd rotation brings the β-subunit back to state i) releasing newly formed ATP.**

**6.** (8 pts) Select **one** of the following **two** questions. Please indicate your choice.

**Choice A:** The aldolase reaction in glycolysis converts fructose-1-6-P to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate. This reaction has a standard energy ($\Delta G^o$) of +24 kJ/M, however during glycolysis the Gibbs free energy, $\Delta G$, is –6 kJ/mol.

i) Briefly describe why there is a large difference between the standard energy and the Gibbs energy during the normal operation of glycolysis.

**Since $\Delta G = \Delta G^o + RT \ln [B]/[A]$, [B] must be much lower than its equilibrium level. This makes the second term negative (by about –30 kJ/mol), giving a Gibbs free energy of –6.**

**This is not an example of direct coupling using ATP hydrolysis. How do you know? Both the substrate (F-1-6-P) and the products have a total of two phosphates. Therefore there is no change in the number of phosphate groups, i.e. ATP has no place to transfer its phosphate.**

**You might imagine a fanciful enzyme that somehow couples the hydrolysis of ATP to the aldolase reaction by conformational changes, similar to ATP synthase, but aldolase is not such an enzyme.**

ii) Is this an example of direct or indirect coupling? *Briefly* justify your answer.

**This is indirect coupling, the concentration of [B] is lowered by a very favorable reaction further on down the pathway. This reaction is not coupled to the aldolase, except that it shares an intermediate in the pathway.**

**Choice B**: The standard energy, $\Delta G^o$, for the reaction: Glucose-6-P $\rightarrow$ Glucose + $P_i$, is – 16 kJ/mol. In glycolysis, the addition of phosphate to glucose, producing glucose-6-P, has a $\Delta G^o$ of –17 kJ/mol.

i) Briefly describe why the $\Delta G^o$ for the phosphorylation of glucose in glycolysis is –17 kJ/mol, not +16 kJ/mol, as you might predict from the $\Delta G^o$ for the release of phosphate from glucose.

**The phosphorylation of glucose is coupled to the hydrolysis of ATP, the net energetics is:**

**+16 kJ/mol – 30 kJ/mol (ATP hydrolysis) = -14 kJ/mol.**

ii) Is the phosphorylation of glucose in glycolysis an example of direct or indirect coupling? *Briefly* justify your answer.

**This is an example of direct coupling, because both the glucose and the ATP bind to the same active site in the enzyme.**

**7.** (10 pts) Please do **one** of the following two choices. Please indicate your choice.

**Choice A:** Select any **one** of the following enzymes and describe how it is regulated by *either* energy sensing *or* hormonal control, whichever is appropriate for the particular enzyme. *Briefly* discuss the physiological/metabolic importance of regulating the enzyme in this fashion.

| Enzyme: | Energy Sensing | Hormonal Control |
|---|---|---|
| Phosphofructokinase (PFK)<br>Active in glycolysis, which produces ATP from glucose. | Activated by AMP or ADP, sensing low energy state of cell. Resulting in the production of ATP from glucose.<br>Inhibited by ATP, glycolysis is turned off when the cell has sufficient ATP. | PFK is activated by F-2-6-P. The levels of this compound are high when glucose is high. Therefore the liver can afford to burn glucose to produce ATP if needed. |
| Fructose-1-6-bisphosphatase<br>Regulatory enzyme in gluconeogenesis, which produces glucose from Pyr, using ATP. | Activated by ATP, sensing high energy state of cell and thus it is possible to produce glucose from pyruvate.<br>Inhibited by AMP, gluconeogenesis is turned off when the cell has does not have sufficient ATP for glucose synthesis. | The phosphatase is inhibited by F-2-6-P. The levels of this compound are high when glucose is high. Therefore the liver does not need to make more glucose, and this pathway is off. |
| Pyruvate dehydrogenase &<br>Citrate syntase.<br>These enzymes are found in the TCA cycle, which produces ATP and NADH | Both inhibited by ATP and NADH. High levels of these compounds indicate high energy reserves in the cell. Therefore the TCA cycle is turned off. | **N.A.** |
| Glycogen Synthase | N.A. | This enzyme is activated when blood glucose levels are high, therefore excess glucose is stored in glycogen. High glucose levels are indicated by the presence of the hormone insulin, which leads to enzyme dephosphorylation, producing the active form of the enzyme. |
| Glycogen Phosphorylase | N.A. | This enzyme is activated when blood glucose levels are low, therefore glucose is released from glycogen. Low glucose levels are indicated by the presence of the hormones glucagon or epinephrine, which leads to enzyme phosphorylation. |

**Choice B:** Briefly describe how a liver cell will respond to **one** of the following hormones. Your answer should briefly discuss the role of the liver in maintaining blood sugar levels and the reason why the hormone is present.

The liver either stores glucose when there is plenty, or releases it when asked.

| Glucagon/epinephrine | Insulin |
|---|---|
| 1. Hormones present when glucose is to be released by the liver.<br>2. Binding of hormones to receptors activates G-protein, Adenyl cyclase is activated,cAMP increases.<br>3. Protein kinases are activated.<br>4. Enzymes become phosphorylated.<br>5. Glycogen phosphorylase is activated, releasing glucose | 1. Hormone is present when glucose is at high levels and should be stored.<br>2. Insulin binding to its receptor leads to activation of phosphatase.<br>3. Phosphate groups are removed from enzymes.<br>4. Glycogen synthase is turned on.<br>5. Glucose is incorporated into glycogen. |

**8.** (12 pts) Please select **one** of the following four questions. Assume T=300 K, RT=2.5 kJ/mol. The formula shown on the right may be useful for questions A-C.

$$\Delta G = \Delta G^0 + RT\ln\frac{[B]}{[A]} \qquad \Delta G = RT\ln\frac{[X]_{IN}}{[X]_{OUT}} + ZF\Delta V$$

$$\Delta G^o = -RT\ln\frac{[B]_{Eq}}{[A]_{Eq}} = -RT\ln K_{EQ} \qquad f_A = \frac{1}{1+K_{EQ}} \qquad f_B = \frac{K_{EQ}}{1+K_{EQ}}$$

**Choice A:** A cell pumps $Na^+$ out of the cell using an enzyme that is a $Na^+$ pump. The concentration of $Na^+$ inside the cell is 0.1M and the concentration outside the cell is 0.15M. The voltage difference across the membrane is 0.1V, with the outside positive. How many ATP molecules are required to pump one $Na^+$ ion? Please show your calculations.

**The above equation for ΔG assumes that the "product" is [X] inside the cell. This has to be modified for this problem since the "product" is $Na^+$ outside the cell:** $\Delta G = RT\ln\frac{[Na^+]_{OUT}}{[Na^+]_{IN}} + ZF\Delta V$ **.**

If you used the equation listed at the top of the page, then it would be necessary to change the sign of the voltage to –0.1V. You would then be calculating the ΔG for movement of Na *into* the cell. So you would have to change the sign of ΔG to obtain the Gibbs free energy for transport out.

**ΔG = 2.5 kJ/mol ln (0.15)/(0.10) + (+1)(96)(+0.1)**

**= +1.0 + 9.6 = + 10.6 kJ/mol.**

**Since ATP hydrolysis releases 30 kJ/mol, one ATP should be sufficient.**

**Choice B:** The standard free energy for the transfer of a 20 residue Gly peptide ($Gly_{20}$) *into* a phospholipid bilayer is +60 kJ/M. The standard free energy for the transfer of the sidechain of Cysteine to a non-polar environment is –3 kJ/mol. You add large amounts of phospholipid to a 1 mM solution of $Cys_{20}$ and allow the system to come to equilibrium. What is the concentration of $Cys_{20}$ free in aqueous solution and what is the concentration of $Cys_{20}$ in the lipid bilayer? Please show your work.

**The overall ΔG° is the sum of the mainchain atoms (Gly) and the sidechain atoms:**

**ΔG° = +60 + (20)(-3) = 0**

**Since ΔG° =0, $K_{EQ}$ =1, and there will be equal amounts of the peptide in the membrane and in solution.**

**Choice C:** A reaction in a metabolic pathway, A → B, has a standard energy, $\Delta G^0$, of +10 kJ/mol. Assuming that the concentration of [A] in the cell is 1 mM, what must the concentration of [B] be to insure that the flux through this step is in the direction A → B? Please show your work.

**To have the flux in the direction from A to B, the Gibbs free energy must be negative. Start by setting it to zero, to calc. $[B]_{eq}$ & then $[B]<[B]_{eq}$.**

**ΔG = +10 + 2.5 ln [B]/(0.001)**

**-4 = ln [B]/(0.001)**

**$e^{-4}$ = [B]/(0.001)**

**0.018 = [B]/(0.001)**

**[B] < 0.000018 M. (18 μM)**

**Choice D:** A *pure* protein of unknown quaternary structure was chromatographed on a gel filtration column. At high protein concentration a single peak, with a molecular weight of 40 kDa, was obtained. At low protein concentrations two peaks were obtained, one with a molecular weight of 20 kDa, and one with a molecular weight of 40 kDa. The same protein was subject to SDS-PAGE and a single band of 20 kDa was obtained. What is the quaternary structure of this protein? Justify your answer and show that it is consistent with *all* of the experimental data.

**The proton contains subunits that are all the same size, 20 kDa. The native molecular weights are 20 kDa and 40 kDa. At high proton concentration, the 40 kDa dimer is formed, at low protein concentrations the 20 kDa monomer begins to form.**