Name _____ Key _____

Chem 352 - Fall 2018 - Exam II

Some potentially useful information:
Ideal gas law constant, $R = 8.314\ J/(mol \cdot K) = 0.08206\ (L \cdot atm)/(mol \cdot K)$
Faraday's constant, $F = 9.469 \times 10^4\ J/(mol \cdot V)$

1. D-Maltose is a disaccharide comprising two molecules of the monosaccharide D-glucose. In solution, D-glucose is present primarily in two pyranose ring forms; 36% in the α-D-glucopyranose form, and 64% in the β-D-glucopyranose form. In solution, D-glucose will convert rapidly between these two ring forms by passing through and open-chain form, which at any instance in time is present in only very small quantities.

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a. In the space below, illustrate this equilibrium reaction using Haworth projections for the α-D-glucopyranose and β-D-glucopyranose structures, and a Fischer projection for the open chain form.

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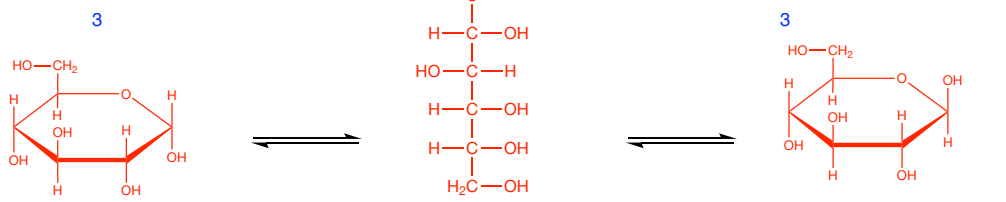

~36% α-D-glucopyranose
Haworth projection

< 1% α-D-glucose
Fischer projection

~64% β-D-glucopyranose
Haworth projection

b. Using Haworth projections, draw the structure of the β-anomer of D-maltose.

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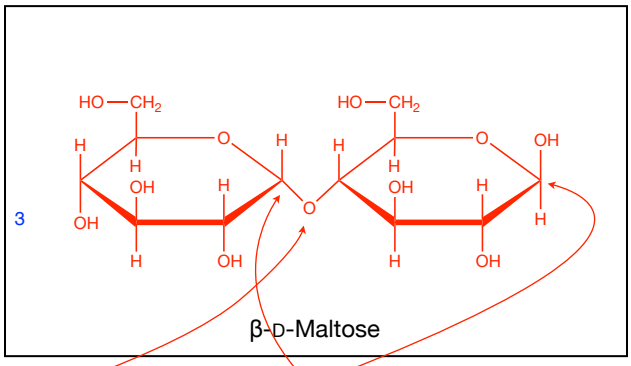

β-D-Maltose

c. While one of the glucose residues in maltose can be found in either its α or β anomer ring form, the other is only found in its α-anomeric ring form. Explain why this is.

5 When forming the glycosidic bond that connects to the to monosaccharides, the anomeric carbon that participates in forming this bond is converted from a hemiacetal to an acetal, locking it into the α-anomer that is no longer able to open and switch to a β-anomer. This is not true of the other monosaccharide, whose anomeric carbon is still a hemiacetal that is free to open and close and and adopt either an α or a β configuration upon reforming the ring.

d. D-Maltose represents the disaccharide unit of a much larger homopolymer of D-glucose. Name this polymer and describe its biological function.

5 The name of the homopolymer is *amylose*, which is a form of starch. Starch is a storage form of glucose that is made by plants. Amylose is a linear polymer of glucose, where each of the glucose residues is connected to the next by an α(1→4) glycosidic bond.

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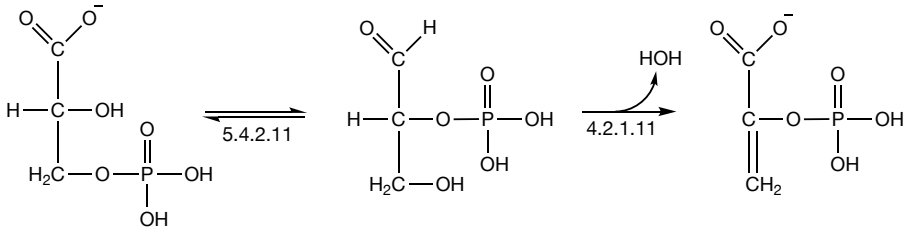
2. Shown below are two consecutive reactions in the glycolytic pathway,



3-phosphoglycerate *2-phosphoglycerate* *phosphoenolpyruvate*

a. The two enzymes that catalyze these reactions are identified above by their Enzyme Commission numbers. Indicate which class of reaction each of enzyme catalyses.

i. E.C. 5.4.2.11 2 Isomerase

ii. E.C. 4.2.1.11 2 Lyase

The kinetics for both these enzymes were studied at an enzyme concentration of 150 nM and the follow values for K_M and V_{max} were obtained from Lineweaver-Burk plots.

	5.4.2.11	**4.2.1.11**
K_M	6.5 mM	43 µM
V_{max}	477 mM/min	72.1 mM/min

b. Which of these enzymes is capable of catalyzing more reactions per second than the other when fully saturated with substrate? 2 5.4.2.11
What is your evidence for this claim?

3 The number of reactions catalyzed by an enzyme per unit time is given by the catalytic rate constant, also called the *turnover number*, k_{cat}. The turnover is determined by dividing the V_{max} value by the concentration of the enzyme used to determine V_{max}. Since the same enzyme concentration was used to determine the V_{max} values for both enzymes, and the V_{max} values share the same units, the enzyme with the greater V_{max} value will be the one with the higher turnover number. This turns out to be the enzyme 5.4.2.11.

c. Which of these enzymes requires a higher concentration of substrate in order to be fully saturated with substrate? 2 5.4.2.11
What is your evidence for this claim?

3 The K_M values can be used to assess an enzyme's affinity for its substrate. The K_M value is equal to the the substrate concentration that produces a half-maximum velocity for the reaction, so the greater the K_M value, the weaker the affinity that na enzyme has for its substrate. Based on this criterium, 5.4.2.11, which requires a substrate concentration of 6.5 mM, as compared to 43 µM (= 0.043 mM) for 4.2.1.11, will require a higher concentration of substrate in order to become fully saturated with substrate.

d. Do one or both of these enzyme demonstrate *catalytic perfection*?

i. 5.4.2.11 (Y/N)? 2 No, 4.2.1.11 (Y/N)? 2 Yes
What is your evidence for these claims?

The way to answer this question is to determine the *catalytic efficiency* for each enzyme in units of 1/M•s, and asking if it is at or near 10^8/Ms. The catalytic efficiency is given as k_{cat}/K_M. It looks like 4.2.1.11 meets this criterium while 5.4.2.11 does not

5.4.1.11: $\frac{k_{cat}}{K_M} = \left(\frac{477\text{x}10^{-3}\ \text{M}}{\text{min}}\right)\left(\frac{1\ \text{min}}{60\ \text{s}}\right)\left(\frac{1}{150\text{x}10^{-9}\ \text{M}}\right)\left(\frac{1}{6.5\text{x}10^{-3}\ \text{M}}\right) = 8.15\text{x}10^{6}\left(\frac{1}{\text{M•s}}\right)$ Which is < 10^8 1/M•s

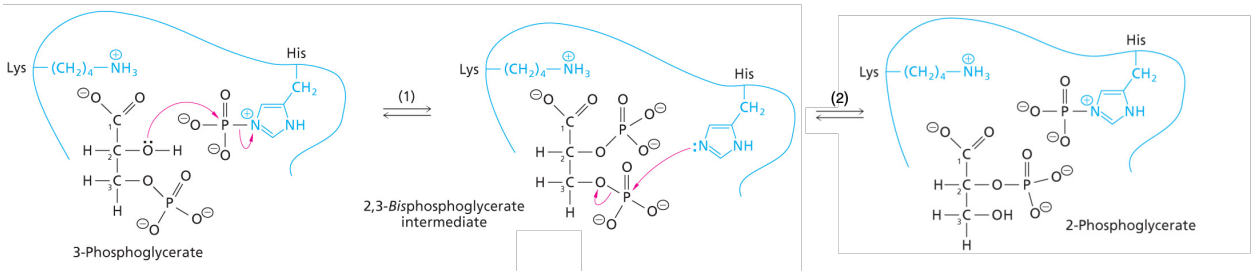
5

4.2.1.11: $\frac{k_{cat}}{K_M} = \left(\frac{72.1\text{x}10^{-3}\ \text{M}}{\text{min}}\right)\left(\frac{1\ \text{min}}{60\ \text{s}}\right)\left(\frac{1}{150\text{x}10^{-9}\ \text{M}}\right)\left(\frac{1}{6.5\text{x}10^{-3}\ \text{M}}\right) = 1.23\text{x}10^{8}\left(\frac{1}{\text{M•s}}\right)$ Which is > 10^8 1/M•s

ii. What does it mean for an enzyme to demonstrate catalytic perfection?

There are only so many improvements that can be made to an enzyme to increase it catalytic efficiency; at some point t rate limiting factor is the rate with which substrate diffuses in to the active site of the
2 enzyme. This occurs when the catalytic efficiency, when expressed as k_{cat}/K_M approaches 10^8 1/M•s.

e. Shown below is a diagram illustrating the catalytic mechanism for the reaction catalyzed by the enzyme 5.4.2.11



i. What catalytic role is being played by the histidine side chain? 2 Covalent bond catalysis
Explain your reasoning:

3 The histidine side chain is making and breaking covalent bonds with the phosphoryl groups, which make up part of both the substrate and the product.

ii. What catalytic role is being played by the lysine side chain? 2 Proximity effect
Explain your reasoning:

3 The lysine side chain is positively charged, and appears to be making a salt-bridge (charge/charge) interaction with the negative charged carboxylate group fo the substrate and product. This interaction appears to help hold and orient the substrate in close *proximity* to the catalytic phosphoryl histidine residue.

3. Draw the structure of the phospholipid *phosphotidylcholine* containing a palmityl (16:0) acyl group at the C1 position, and a linoleyl (18:2, *cis*-$\Delta^{9,12}$) acyl group at the C2 position.

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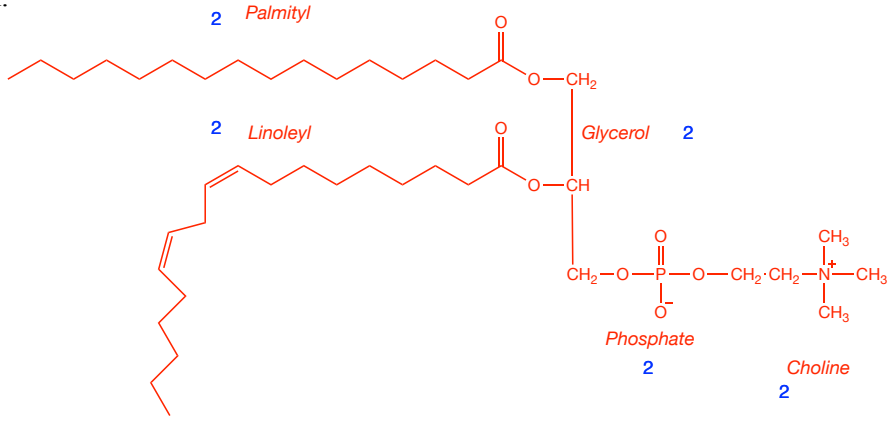
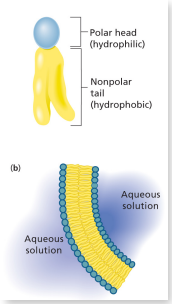


a. In a couple of sentences, describe the structure that forms spontaneously when phosphotidylcholine is mixed with water. Include in this description a mention of the intermolecular interactions that lead to the formation of this structure.

5 Phospholipids, such as phosphotidylserinecholine, are highly *amphipathic*, meaning they contain a moiety that is very *hydrophobic*, along with a moiety that is at the same time very *hydrophilic*. When placed in water these molecules will aggregate in away that satisfies both of these characteristics. Phospholipids form lipid bilayers, in which the surfaces of the bilayer expose the hydrophilic moieties to water, while at the same time burying the hydrophobic regions on the inside of the bilayer and away from water. Once formed, the bilayer structure is then stabilized by the favorable hydrogen bonding interactions between the exposed choline phosphate head group and the water, and the van der Waals interactions between the fatty acid.side chains.



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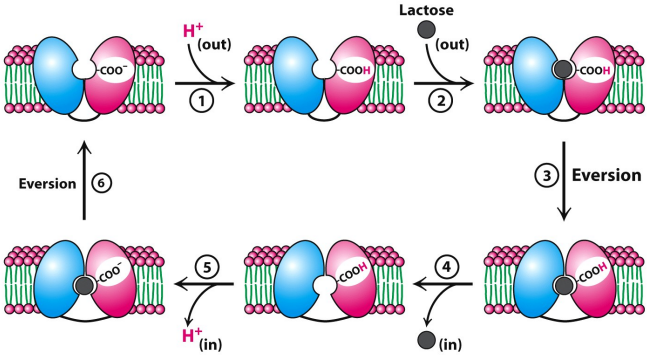
4. *Lactose permease* is an integral membrane protein that transports lactose across bacterial cell membranes along with hydrogen ions. The transport cycle is illustrated in the diagram below.



2 Symport (The lactose and H+ are both being transported in the same direction, from [out] to [in].

a. What kind of transport is this an example of? *uniport*, *symport* or *antiport*? same direction, from [out] to [in].

b. Given the membrane potential $\Delta\Psi$ (Ψ_{in} - Ψ_{out}) is -65 mV, is the transport of lactose into the cell favorable at 37°C if on the outside the *pH* is 6.4 and the concentration of lactose is 0.035 mM, while on the inside the *pH* is 7.6 and the concentration of lactose is 1.00 mM? 2 It is favorable

Give your evidence for this claim: In this transport system, the transport of lactose into the cell is coupled to the simultaneous transport of hydrogen ions into the cell. The overall free energy change (ΔG) is given by the sum of the free energy changes for each.

$$\Delta G = \left(RT\ln\left(\frac{[\text{lac}]_{in}}{[\text{lac}]_{out}}\right) + z\mathcal{F}\Delta\Psi\right)_{\text{lactose}} + \left(RT\ln\left(\frac{[H^+]_{in}}{[H^+]_{out}}\right) + z\mathcal{F}\Delta\Psi\right)_{H^+}$$

$$= \left(\left(8.314\ \frac{J}{mol\cdot K}\right)(310\ K)\ln\left(\frac{1.0\times10^{-3}\ M}{0.035\times10^{-3}\ M}\right) + (0)\left(9.646\times10^{4}\ \frac{J}{mol\cdot V}\right)(-0.065\ V)\right)_{\text{lactose}} + \left(\left(8.314\ \frac{J}{mol\cdot K}\right)(310\ K)\ln\left(\frac{10^{-7.6}\ M}{10^{-6.4}\ M}\right) + (+1)\left(9.649\times10^{4}\ \frac{J}{mol\cdot V}\right)(-0.065\ V)\right)_{\text{lactose}}$$

5 $$= \left(8{,}640\ \frac{J}{mol} + 0\ \frac{J}{mol}\right)_{\text{lactose}} + \left(-7{,}121\ \frac{J}{mol} - 6{,}272\ \frac{J}{mol}\right)_{H^+} = \left(8{,}640\ \frac{J}{mol}\right)_{\text{lactose}} + \left(-13{,}393\ \frac{J}{mol}\right)_{H^+} = -4{,}753\ \frac{J}{mol}$$

The transport of lactose is active, because on its own the transport is unfavorable (ΔG = 8,640 J/mol). By coupling its transport to the favorable transport of H+ across the membrane (ΔG = -13,393 J/mol), the overall transport is now favorable (ΔG = -4,753 J/mol).

c. If the transport of lactose into the cell is favorable, is this is this an example of *active* or *passive* transport? 2 active

4 Explain: The transport of lactose is active, because on its own the transport is unfavorable (ΔG = 8,640 J/mol. By coupling its transport to the favorable transport of H+ across the membrane, the overall transport is now favorable. Because the energy source for this transport is not direct, but instead coupled to the simultaneous transport of H+ ions across the member, this is considered secondary active transport.

d. What class of biological molecule, *e.g.*, *amino acid*, *carbohydrate*, *lipid*, *etc.* does lactose belong to? 2 Carbohydrate

e. Draw the chemical structure for α-D-lactose.

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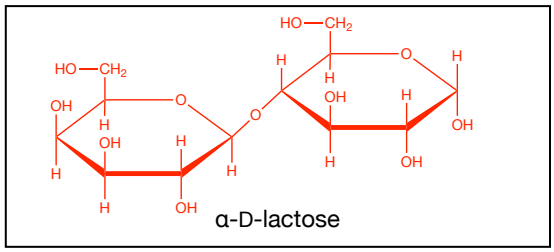

f. What two monosaccharides comprise α-D-lactose?

2 β-D-galactopyranose

2 α-D-glucopyranose

g. How many chiral carbons does α-D-lactose possess? 2 10

h. What type of *glycosidic bond* connects the two monosaccharides? 2 β(1→4)

100