C483 Exam 2
Fall 2016

Name Key Seat Number _____

Student ID _____ Discussion section: M R

The last page of this exam contains equations, data, and scratch area.
This exam contains 110 points on 11 pages. The highest score you may earn on this exam is 100 points.

1 _____/20pts

2. _____/10pts

3. _____/20pts

4. _____/10pts

5. _____/10pts

6. _____/10pts

7. _____/10pts

8. _____/10pts

9. _____/10pts

Total:

Regrading: All requests for regrades must be submitted in writing within 48 hours of the return of the exam. You must explicitly state what has been misgraded and why it is an error. The entire exam will be regraded, which could result in points being added or deducted overall.

Section 1: Reading guides (50 points)

1. 20 pts. Fill in the blanks (2 points each.)

A. A low (high/low) value of K_M (k_{cat}/K_M) suggests a strong affinity of enzyme for the substrate.

B. Allosteric or multi-substrate enzymes do not fit the Michaelis-Menton model of enzyme kinetics.

C. Transition State analogs typically bind to enzyme more tightly than substrate analogs, and often serve as irreversible inhibitors.

D. AMP or ADP is a positive effector for phosphofructokinase, which makes sense physiologically because it is a signal to produce more ATP.

E. Cholesterol is the main compound found in animal cells that helps maintain the right level of membrane fluidity of a range of temperatures.

F. The two most common types of membrane bound receptors in signal transduction are G-protein coupled receptors (7-TM) and receptor tyrosine kinase.

G. A mutated gene leading to cancer is called an oncogene. An example is the gene encoding *Ras*, a small G-protein.

H. The C-2 epimer of D-glucose is called D-mannose.

I. The proteasome allows cytosolic proteins no longer needed to be safely hydrolyzed in the presence of other cytosolic proteins that must remain intact.

J. Irreversible reactions have a large negative free energy, but metabolic reactions with free energy close to zero are termed near-equilibrium reactions.

2. 10 pts. Write True or False (1 points each)

A. False A hyperbolic curve on a plot of [P] vs. time is indicative of saturation kinetics.

B. True Values of V_{max} and K_M can be determined graphically from a Lineweaver-Burk plot.

C. True Lateral diffusion of phospholipids in a lipid bilayer is relatively fast.

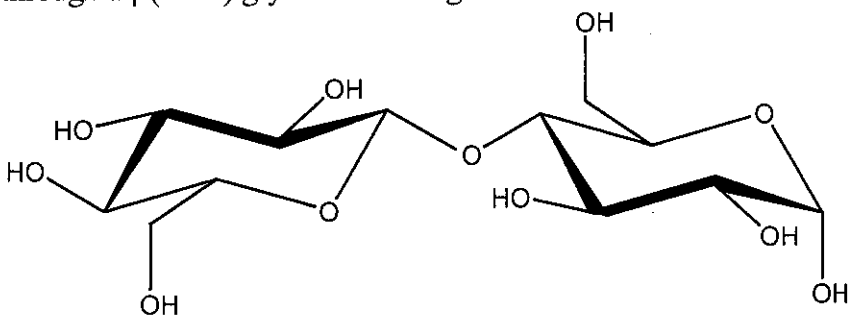
D. False In the β-adrenergic receptor signal transduction pathway, the second messenger cAMP is "turned off" by being transformed back to ATP.

E. True Sphingomyelins are sterically similar to their glycerophopholipid counterparts, but are built on a different backbone.

F. False Voltage-gated channels allow ions to flow against their concentration gradients when opened by a change in membrane potential.

G. True A phosphodiesterase is involved in turning off the signal of the β-adrenergic receptor by destroying the second messenger produced in the pathway.

H. True The disaccharide shown below is made up of two glucose molecules linked through a β(1→4) glycosidic linkage.



I. True In the intestinal cell, fatty acids must be reassemble into triacylglycerides before transport to long-term storage, but sugars do not need to be reassembled into polysaccharides prior to transport to long-term storage.

J. False The standard free energy of a given reaction depends upon both the inherent stability of the bonds and the concentrations of the reactants and products in a given situation.

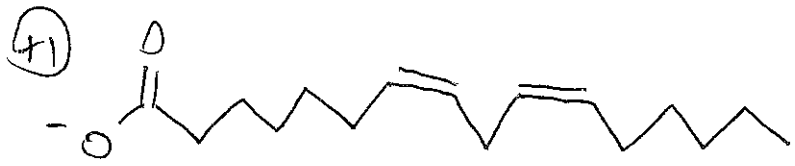
3. 20 pts. Short answer (5 points each)

A. Why is Tylenol toxic at large doses?

(+1) - P450 enzyme in liver oxidizes tylenol

(+4) - At high dosage, the oxidized form spontaneously reacts to form a toxic substance at significant concentration

B. Draw the structure of a 16:2 *n*-6 fatty acid.



(+1) 16 C

(+1) two d.b.

(+1) cis

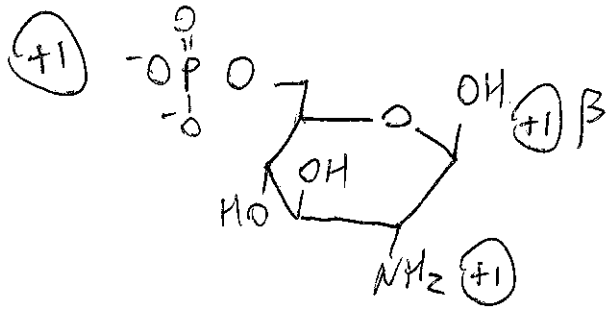
(+1) right position

C. Initially, a nerve cell has a membrane potential of -70 mV with a concentration of 150 mM outside the cell and 12 mM inside the cell. What is the membrane potential after enough sodium has travelled into the cell to raise the concentration inside the cell to 40 mM? (Assume the membrane potential change is only affected by sodium ions, and the concentration of sodium outside the cell remains about constant.)

$$\psi = \frac{RT}{2F} \ln \frac{40\,mM}{150\,mM} = \frac{8.314\,(310K)}{96485\,J/mol \cdot V} \ln \frac{.040}{.150}$$

$$\psi = -35\,mV$$

D. Glucosamine is the compound produced when the C-2 hydroxyl of glucose is replaced with an amino group. Draw the β-anomer of D-6-phosphoglucosamine

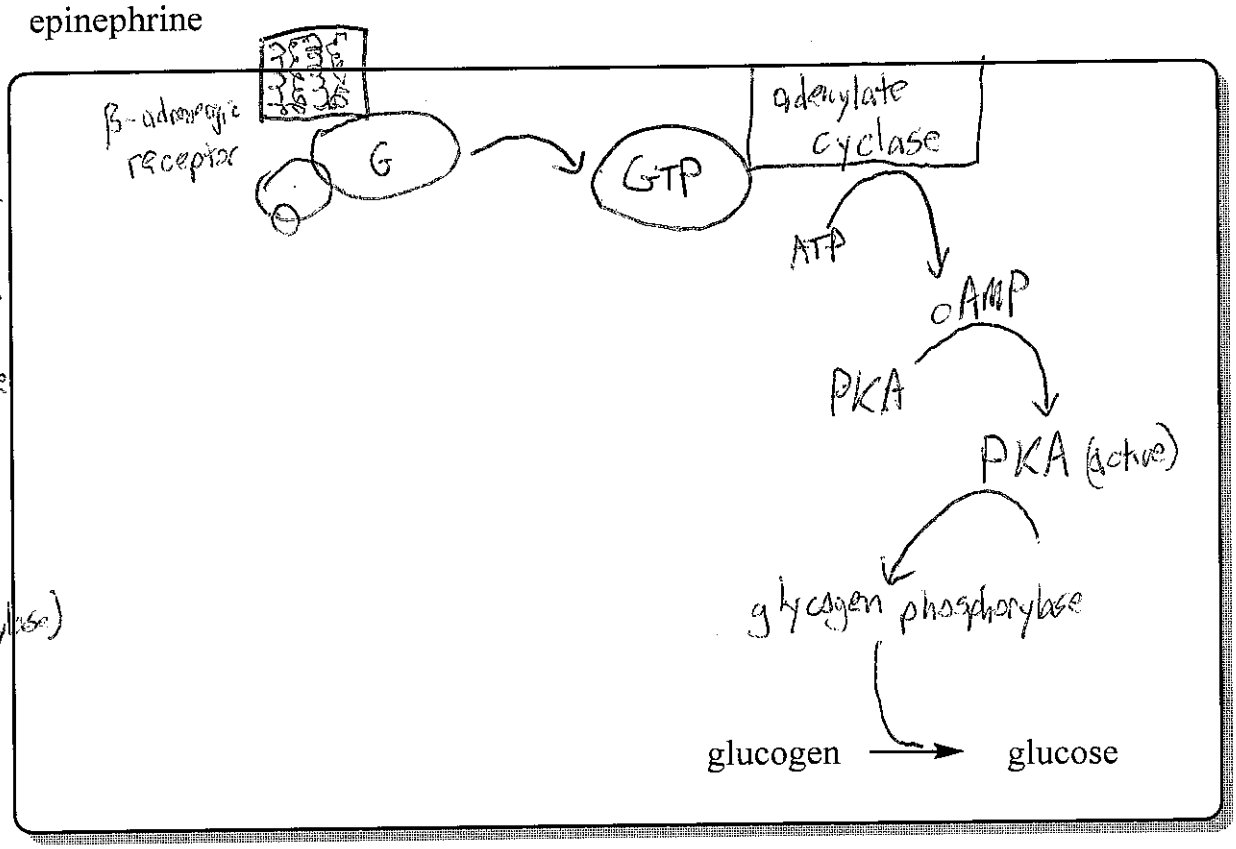


(+1) β

(+2) pyranose with stereochem

Section 2: Problems (10 points each)

4. Draw a basic schematic for the signal transductin pathway of the β-adrenergic receptor. Include the major components in the muscle cell below that lead to the release of glucose from glycogen, starting with epinephrine.



1. ligand
1) receptor
+1) G-protein
-1) Adenylate cyclase
+1) cAMP
-1) PKA
-1) effector (phosphorylase)

Refer to the figure you drew to indicate one instance of signal aplification.

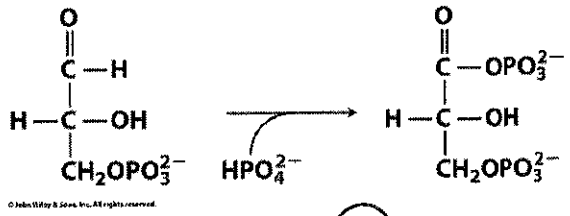
Any of the following: activation of G-protein
formation of cAMP
Kinase cascade

+2

How is the transducer in this pathway "turned off?"

+2 G-protein hydrolyzes its bound GTP. The conformational change

5. 1,3-bisphosphoglycerate is made in glycolysis according to the reaction below. How many high energy bonds are present in the starting material? How many high energy bonds are present in the products? Explain why the standard free energy for this reaction is not very far from zero using qualitative principles.



+1 zero HE bonds +1 one high energy bond

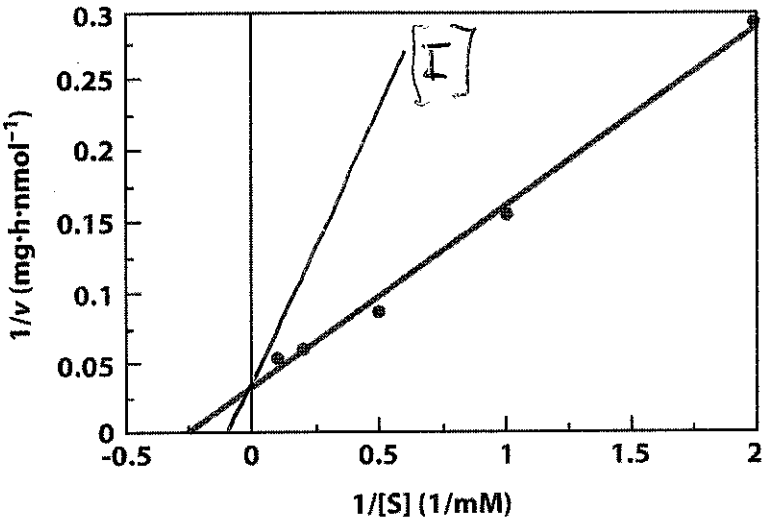
+4 Although this rxn is uphill in formation of a high energy bond, it is an oxidation (which is downhill) with the net $\Delta G^{\circ\prime} \approx$ zero.

A key reaction in carbohydrate metabolism involves the transfer of a phosphate group from 1,3-bisphophoglycerate to ADP to form 3-phosphoglycerate and ATP. Using appropriate data from the end of the exam, what is the standard free energy of this transfer? Write out the reactions you used to determine this standard free energy.

+4

	$\Delta G^{\circ\prime}$
1,3 bisphosphoglycerate → 3-phosphoglycerate + P_i	$-49.4 \frac{kJ}{mol}$
ADP + P_i → ATP	$+30.5 \frac{kJ}{mol}$
1,3bPG + ADP → 3PG + ATP	$\Delta G^{\circ\prime}$ $-18.9 \frac{kJ}{mol}$

6. The kinetics of transport through protein transporters can be described using the language of Michaelis and Menten. Below is a double reciprocal plot for glucose by the pericyte transporter.



A. How would you use this graph to determine the transporter's affinity for glucose? Estimate a numerical answer using the graph.

(+3) Affinity is analogous to K_m. On this graph, it is the x-intercept.

$-\frac{1}{K_m} = -0.25\ mM^{-1}$

$K_m = 4\ mM$

B. How would you use this graph to quantitate the transporting ability of the transporter? Estimate a numerical answer from the graph.

(+3) The transport ability would be analogous to K_{cat}. Without [Transporter], this cannot be determined exactly, but it is proportional to V_{max}. In this case, $V_{max} = \frac{1}{y\text{-intercept}} = \frac{1}{.033} = 30\ \frac{nmol}{mg \cdot h}$

(+4) C. On the graph above, draw the double reciprocal curve you would expect if a transport inhibitor that was competitive with glucose binding were added.

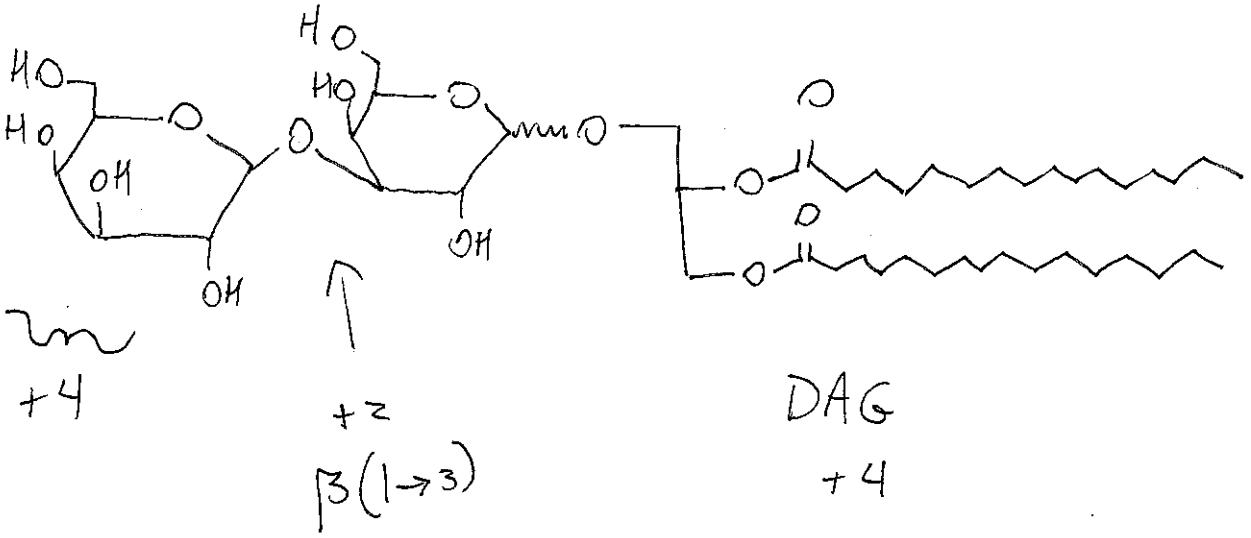
7. A new glycolipid has been discovered. The following facts are known about this glycolipid.

   A. It fits well into lipid bilayers.

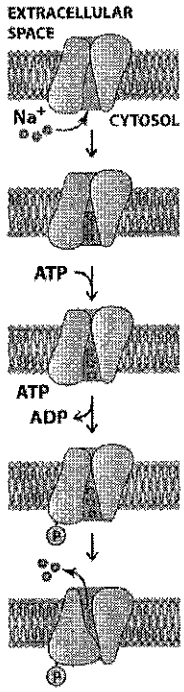
   B. When it is hydrolyzed, the following are isolated: glycerol, two equivalents of 16-carbon saturated fatty acids, and two equivalents of galactose. No phosphate is released.

   C. One galactose residue can be hydrolyzed off of the glycolipid by an enzyme that cleaves β(1→3) glycosidic linkages of D-galactose.

Draw the structure of a glycolipid that fits all of these data:



8. Write a verbal mechanism for the first stage of sodium/potassium antiporter. Indicate which, if any, of the first four steps are irreversible.



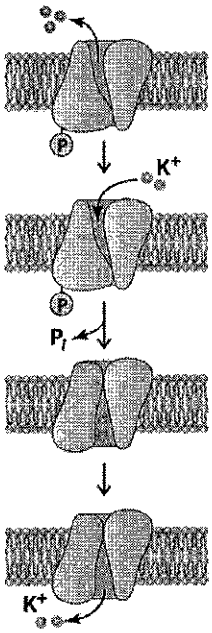
(1) Na^+ from inside cell binds to transport, which (+1)

(2) allows ATP to bind to the transporter (+1)

(3) ATP phosphorylates the transporter (+1) irreversibly (+1)

(4) change in conformation of phosphorylated transporter releases Na^+ outside of cell (+1)

Write a verbal mechanism for the second stage of sodium/potassium antiport. Indicate which, if any, of the last three steps are irreversible.

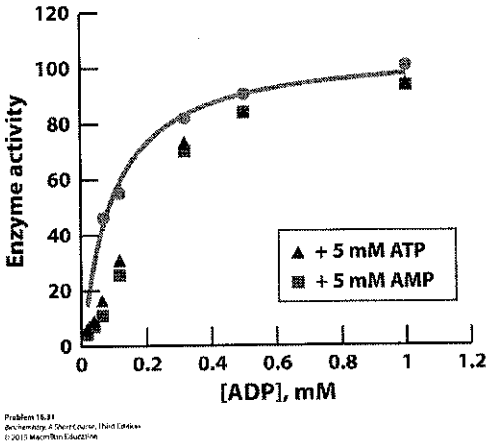


(5) K^+ from outside of cell binds to transporter (+1)

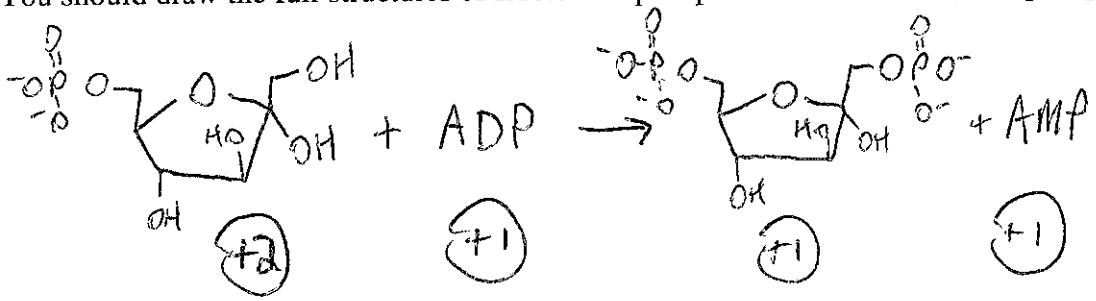
(6) which causes hydrolysis of phosphorylated transporter (+1) which causes conformational change (+1) irreversibly (+1)

(7) and K^+ is released inside of cell. (+1)

Section 3: Case study (10pts) An unusual kinase has been isolated from a thermal vent bacterium, which transforms fructose-6-phophate into fructose-1-6-bisphosphate. The kinase is unusual in the fact that, typically, ATP is the phosphoryl group donor for a kinase, but in this case, ATP is not a substrate. Refer to the data below to answer the questions.



A. Write the full equation for this kinase-catalyzed reaction including all substrates and products. You should draw the full structures of fructose-6-phosphate and fructose-1,6-bisphosphate.



(+2) (+1) (+1) (+1)

(Straight chain is acceptable)

B. ATP is an allosteric effector of this reaction. How is this supported by the data?

(+2) Addition of 5mM ATP (triangles) shifts the curve to the right in a sigmoidal shape.

C. Is ATP an allosteric activator or inhibitor? Explain. If this enzyme is a regulatory enzyme in the pathway that produces ATP, explain the logic of this regulation.

(+1) Inhibitor

(+2) In the presence of a large amount of ATP, the pathway that makes ATP should be shut off. It acts as a type of feedback inhibitor

Data Tables and scratch work

TABLE 12-4

Standard Free Energy Change for Phosphate Hydrolysis

Compound	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
ATP → AMP + PP_i	−45.6
Phosphocreatine	−43.1
ATP → ADP + P_i	−30.5
Glucose-1-phosphate	−20.9
PP_i → 2 P_i	−19.2
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

$\Delta G = RT \ln \frac{[X]in}{[X]out} + ZF\Delta\psi$ $\Delta\psi = \frac{RT}{ZF} \ln \frac{[X]in}{[X]out}$

R = 8.314 J/ mol · K

F = 96,485 J/V · mol

$$\Delta G_{reaction} = \Delta G^{\circ\prime}_{reaction} + RT \ln \frac{[products]}{[reactants]}$$