C483 Exam 2
Spring 2016

Name Key Seat Number _____

Student ID _____ AI _____

The last page of this exam contains information you might find useful.
This exam contains 110 points. 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. The inhibition of phosphofructokinase by phosphoenolpyruvate is an example of feedback inhibition because phosphoenolpyruvate is produced in one of the last steps of a pathway.

B. Write the Michaelis-Menton equation below:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

C. k_{cat} is also known as the turnover number.

D. Draw an omega-3 fatty acid with 16 carbons at physiological pH.

E. The favorable movement of an ion across a membrane depends on both concentration and membrane potential.

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

G. Starch and cellulose differ only in the stereochemistry of the glycosidic bond.

H. A five-carbon sugar with a carbonyl on carbon 1 could be called an aldopentose.

I. An example of a two-electron reducing cofactor is NADH ($FADH_2$).

J. Unneeded proteins are marked for desctruction by ubiquitin and then hydrolyzed in the proteasome.

2. 10 pts. True or false (1 points each)

A. True The shape of a velocity vs. [E] curve when the concentration of substrate is kept constant is linear.

B. False In general, a transition state analog is a better uncompetitive inhibitor than a substrate analog.

C. True A noncompetitive inhibitor will reduce the V_{max} of the enzyme, but the K_M value will remain the same.

D. True Lateral diffusion of a phospholipid through a bilayer is faster than transverse diffusion through a bilayer.

E. False Integral proteins can be reversibly bound to the membrane through a lipid anchor.

F. False A potassium channel is able to exclude the smaller sodium ion through unfavorable steric interactions.

G. False Sucrose is a non-reducing sugar because it contains a hemiacetal functional group that is in equilibrium with its open-chain form.

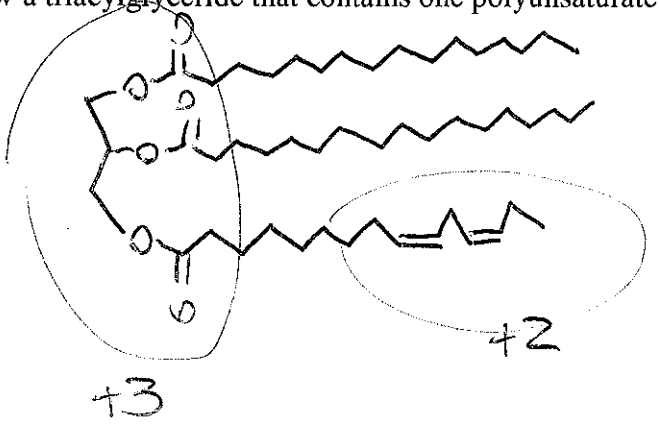
H. True It is possible to make an unfavorable reaction occur spontaneously by coupling it with a favorable reaction.

I. True The direction of spontaneity for a near-equilibrium reaction depends on the relative concentrations of the reactants and products.

J. False Dietary starch is first degraded by amylase in the saliva, then by pancreatic proteases in the intestine before being carried through the blood to muscle and liver for storage.

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

A. Draw a triacylglyceride that contains one polyunsaturated fatty acid.



B. The concentration of calcium ion in the endoplasmic reticulum (outside) is 1 mM, and the concentration of Ca^{+2} in the cytosol is 0.1 mM. Calculate the difference in free energy at 37 °C assuming a membrane potential of + 50 mV.

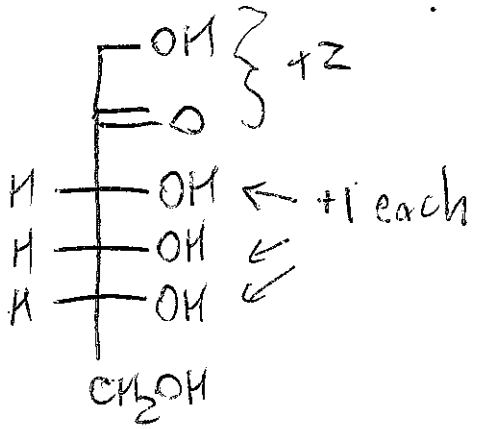
accept 298K

$$\Delta G^\circ = 8.314 \frac{J}{mol\,K}(310\,K)\ln\frac{1\times10^{-4}}{1\times10^{-3}} + (2)(96485)(0.050\,V)$$

$$= -5.93\,KJ + 9.648\,KJ$$

$$= +3.72\,KJ$$

C. Draw a Fisher projection of the product of an isomerase catalyzed reaction of the C-3 epimer of glucose.



D. A new enzyme called C483ase has recently been discovered. Tests conducted on it using a 0.1nM solution of enzyme gave a V_{max} of 2.5×10^{-8} M/s and a K_m of 3.7×10^{-5} M. Calculate the catalytic efficiency of this enzyme.

$$V_{max} = [E]_T K_{cat}$$

$$K_{cat} = \frac{2.5\times10^{-8}\,\frac{M}{s}}{0.1\times10^{-9}\,M} = 250\,s^{-1}$$

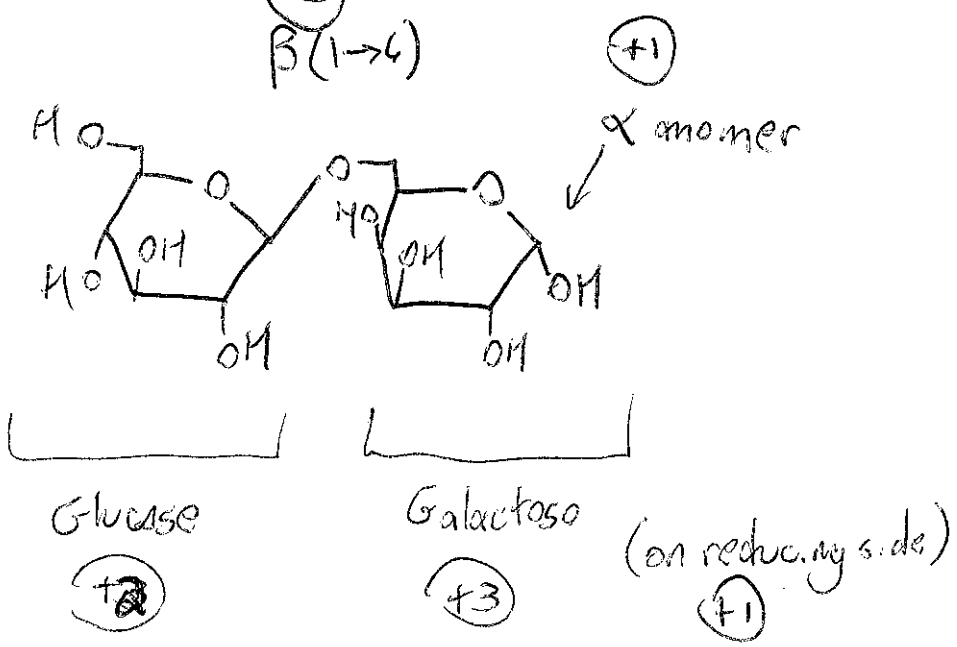
$$\frac{K_{cat}}{K_M} = \frac{250\,s^{-1}}{3.7\times10^{-3}\,M} = 6.8\times10^{6}\,\frac{1}{M\cdot s}$$

Section 2: Problems (10 points each)

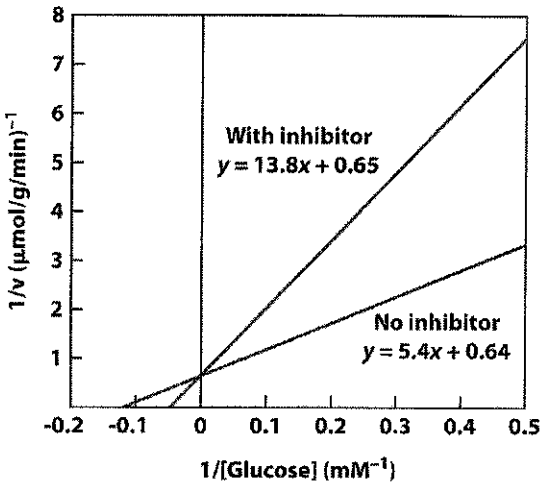
4. Fill in the blanks for this description of the β-adrenergic receptor pathway:

The β-adrenergic receptor is structurally a 7-TM receptor, allowing it to span the membrane. Inside the membrane, it binds to a transducer called a G-protein. This inactive transducer will bind to the receptor only when the receptor is bound to its ligand, called epinephrin. The inactive transducer will then release GDP and bind GTP to become active. The active transducer binds to an enzyme called adenylate cyclase which will produce cAMP. cAMP is a type of second messenger like DAG or PIP_2. At high concentrations, cAMP will bind to PKA, which begins a kinase cascade. In this cascade, proteins are activated or deactivated by being phosphorylated by kinases. These activated/deactivated proteins can then be set back to their original level of activity by the action of the opposing enzymes, which are phosphatases.

5. A new amylase substrate has been developed, which is a disaccharide of galactose and glucose linked via a β (1→6) linkage. The galactose residue is on the reducing end of the disaccharide. Draw the structure of its α-anomer. +3



6. Unidirectional glucose transport into the brain was measured in the presence and absence of phlorizin. The velocity of transport was determined at various glucose concentrations and is shown as a Lineweaver Burk plot below:



A. Calculate K_M for this process in the presence and absence of this inhibitor. Show all work.

(+4)

$K_m = -\frac{1}{x_{int}}$ $\quad x = \frac{-.64}{5.4} = -0.12\ mM^{-1}$

$K_m = 8.3\ mM$

With inhibitor: $\quad x = \frac{-.45}{13.8} = -0.047\ mM$

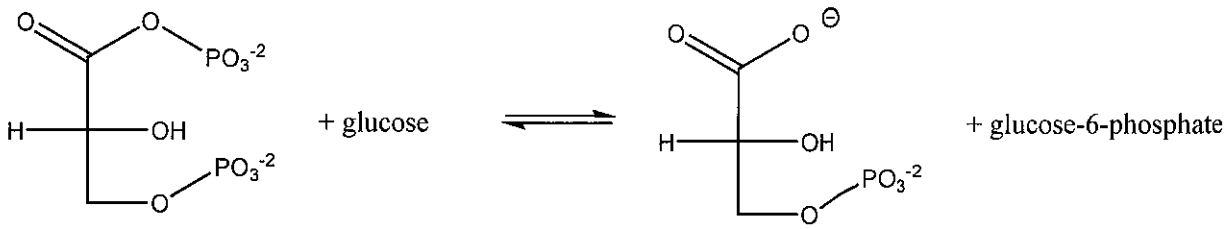
$K_m = 21.2\ mM$

B. Would this inhibitor be more or less effective under high [glucose]? Explain with reference to the glucose transporter.

(+4) It is a competitive inhibitor, so it would be less effective at high [glucose.]

(+2) Glucose would out-compete the inhibitor to bind to the glucose transporter.

7. Based on structural principles would you expect the reaction below to be spontaneous, nonspontaneous, or in between? Explain.



1,3-bisphosphoglycerate

3-phosphoglycerate

(+3) Spontaneous - a high energy bond (phosphoanhydride) is broken and a lower energy phosphate ester is made

Using data from the last page, is this reaction spontaneous or not under cellular conditions in which [1,3-bPglycerate] is 1 mM, [3-phosphoglycerate] is 0.1 mM, [glucose] is 4 mM, and [glucose-6-phosphate] is 1 mM?

1,3 bisPglycerate $\longrightarrow$ 3 phosphoglycerate + P_i $\Delta G^{\circ\prime} = -49.4$

Glucose + P_i $\longrightarrow$ G-6-P $\Delta G^{\circ\prime} = +13.8$

(+3) $\Delta G^{\circ\prime} = -35.6 \frac{KJ}{mol}$

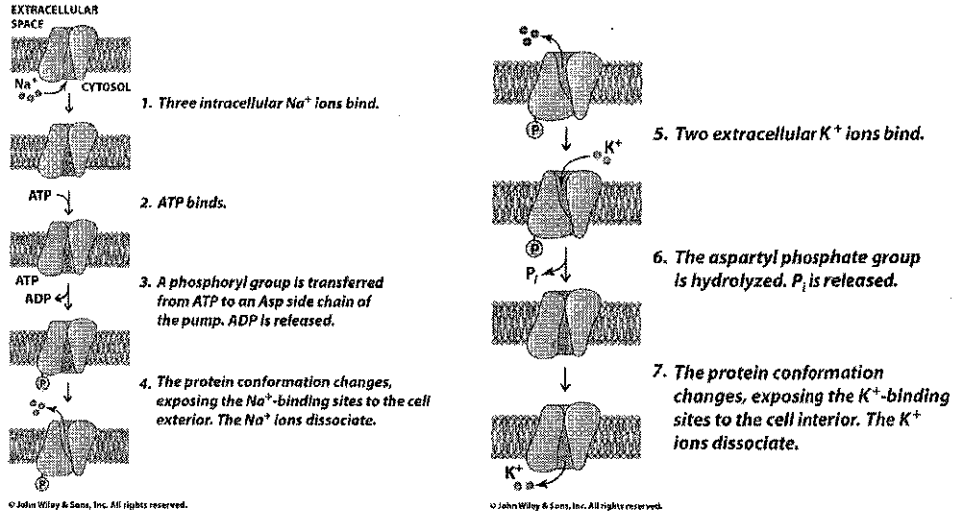
(+3) $\Delta G = \Delta G^{\circ} + RT \ln \frac{[P]}{[R]}$

$= -35.6KJ + 8.314 (298K) \ln \frac{[0.1 \times 10^{-3}][1 \times 10^{-3}]}{[4 \times 10^{-3}][1 \times 10^{-3}]}$ (or 310K)

$= -35.6KJ + -9.1 KJ$

$= -44.7 \frac{KJ}{mol}$ spontaneous (+1)

8. A picture mechanism of the sodium-potassium ATPase is shown below. Answer questions based on this mechanism.



A. Is this transporter a symporter, antiporter, or neither? Explain.

(+3) Antiporter - Na^+ must go out for K^+ to come into cell

B. Which step(s) explain why this pump establishes sodium and potassium gradients rather than allowing the ions to flow in the passive direction?

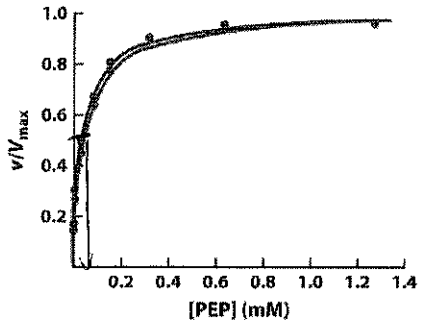
(+3) Step 3 - ATP hydrolysis irreversibly opens Na^+ out of the cell

Step 6 - hydrolysis of aspartyl phosphate pumps K^+ into cell

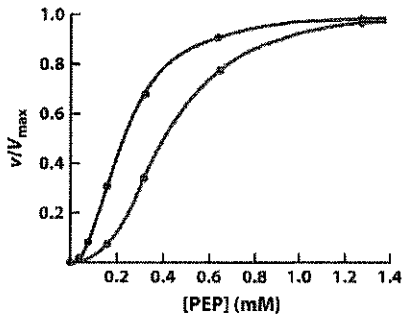
C. If excess potassium where to be injected into the cell, how would this affect the cell's ability to pump out sodium—would it increase, decrease, or remain unchanged? Explain.

(+4) Thermodynamically, it would decrease the cell's ability. By making a greater gradient, it would become thermodynamically unstable to create a greater K^+ gradient, and it is linked to Na^+ gradient through the antiporter.

Section 3: Case study (10pts) Pyruvate kinase is a multimeric protein that catalyzes the last step of a catalytic pathway, the reaction of phosphoenolpyruvate (PEP) with ADP to make ATP and pyruvate. It has four isozymes, some regulated, and others unregulated. The two graphs below investigate the effect of Fructose-1,6-bisphophate (F1,6bP) on the activity of one of the wildtype isozymes (Graph A) and a mutant form of the isozyme in which Ala398 has been changed to Arg (Graph B.) In graph A, the curves with and without F1,6bP overlap; in Graph B, the curve with F1,6bP present is to the left of the curve with no F1,6bP.



A.



B.

A. Is the wild type pyruvate kinase an allosteric enzyme? Explain with reference to data.

(+2) Not allosteric - graph A is not sigmoidal.

B. What is K_M for the wild type enzyme in the presence of F1,6bP and without F1,6bP? Explain with reference to data.

(+3) K_m for wild type is less than 0.1 mM both with and without effector.

C. What is V_{max} for the mutant enzyme? Explain with reference to data.

(+2) V_{max} cannot be determined because it is a relative graph - $\frac{V}{V_{max}}$ has a maximum of 1.0.

D. Is F1,6bP a negative effector or a positive effector? Explain with reference to data.

(+3) It is a positive effector for the mutant. Shifting the curve to the left decreases K_m, makes it more active at lower [PEP].