Biochemistry 463, Summer II **Your Name:** _____

University of Maryland, College Park **Your SID #:** _____

Biochemistry and Physiology **Prof. Jason Kahn**

Exam II (100 points total) **August 5, 2013**

You have 80 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be concise and clear. I have given you more space than you should need. There is extra space on the last page if you need it.

You will need a calculator for this exam. No other study aids or materials are permitted.

Partial credit will be given, *i.e.*, if you don't know, guess.

Useful Equations:

$\Delta S_{system} - \Delta H_{system}/T \geq 0$ $\quad pH = -\log([H^+])$ $\quad E = mc^2$

$S = k\ln W$ $\quad \Delta G = \Delta H - T\Delta S$ $\quad pH = pK_a + \log([A^-]/[HA])$

$K_a = [H^+][A^-]/[HA]$ $\quad \Delta G° = -RT\ln K_{eq}$ $\quad e^{i\pi} + 1 = 0$

$$v_0 = \frac{(1/\alpha')V_{max}[S]}{(\alpha/\alpha')K_M + [S]}, \text{where } \alpha = 1 + \frac{[I]}{K_I} \text{ and } \alpha' = 1 + \frac{[I]}{K_I'}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \quad v_0 = \frac{V_{max}[S]}{K_M + [S]} \quad K_M = \frac{k_{-1} + k_2}{k_1}$$

Honor Pledge: At the end of the examination time, please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

1. (28 pts) Michaelis Menten Kinetics

(a; 4 pts) We used the Steady State Approximation and the conservation of total enzyme concentration in deriving the Michaelis-Menten equation. Write down equations for the SSA and the conservation of enzyme.

Score for the page_____

(b; 6 pts) Do you need to know E_T (total enzyme) to determine (circle Y or N for each): K_m (Y/N)? V_{max} (Y/N)? k_{cat} (Y/N)? Why do the estimated values for k_{cat} frequently increase as an enzyme is studied more intensively?

(c; 10 pts) Sketch a Lineweaver-Burke plot for an enzymatic reaction performed at increasing concentrations of a pure competitive inhibitor. Pure competitive inhibitors tend to resemble (circle one): S, P, or the TS.

Score for the page_____

(d; 8 pts) Consider the MM equation at low substrate concentration to explain why top-performing enzymes all have similar k_{cat}/K_m values even though their individual k_{cat} and K_m parameters vary widely. What is the operational definition of "low substrate concentration" in this context?

2. (33 pts) Mechanisms

(a; 6 pts) Draw the business end of TPP. What is one of its mechanistic functions in enzymatic catalysis? Many enzymes use metal ions in their active sites. Give a common mechanistic function for Zn^{++} or Mg^{++} in active sites.

Score for the page_____

(b; 12 pts) Draw the mechanism for the aldolase reaction, which converts F(1,6)BP to GAP + DHAP. You don't need to remember any residue numbers, just indicate the active site residues as Lysine and as acids and bases. What is the function of the Schiff's base in this mechanism?

Score for the page_____

(c; 15 pts) Write down reaction catalyzed by pyruvate carboxylase and name the cofactor used. Include all reactants and products except protons and water. You do not need to draw the mechanism. Explain how pyruvate carboxylase is activated by a feed-forward mechanism and the biochemical rationale for this. Name the enzyme that channels oxaloacetate into gluconeogenesis.

3. (39 pts) Regulation

(a; 6 pts) Why do muscle cells convert pyruvate to lactate, which is essentially a metabolic dead end that just leads back to pyruvate? Refer back to a specific previous step in glycolysis in your answer.

Score for the page_____

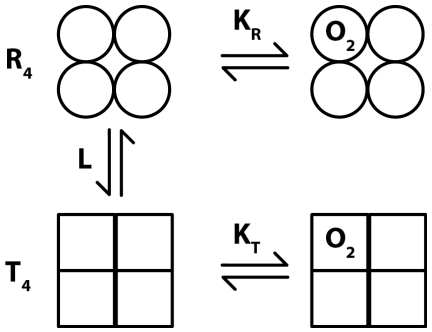
(b; 12 pts) Sketch the Cori cycle. Why do liver cells express glucose-6-phosphatase whereas muscle cells do not? Muscle cells do not have glucagon receptors. In terms of the glycolysis vs. gluconeogenesis switch, why don't they need them?

(c; 4 pts) Why do liver cells express a hexokinase isozyme (glucokinase) with a much higher Km than muscle hexokinase?

(d; 5 pts) Glucose -> G6P fits at least one criterion for being a useful regulated step. Name the criterion, and give one reason this step isn't it highly regulated.

Score for the page_____

(e; 12 pts) Hemoglobin allostery. The states in the symmetry model for hemoglobin allostery are R_4 and T_4 tetramers with various numbers of oxygens bound. Complete the sketch below to show the multiple thermodynamic cycle argument that shows that each successive oxygen binding event causes a stronger and stronger preference for the R state.



Page	Score
1	/4
2	/16
3	/14
4	/12
5	/21
6	/21
7	/12
Total	**/100**

Score for the page_____