Biochemistry 463, Summer II **Your Name:** - Key -

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.

$\frac{d[ES]}{dt} = 0$ (+2) $\qquad E_T = [E] + [ES]$ (+2)

Score for the page_____

+1 each

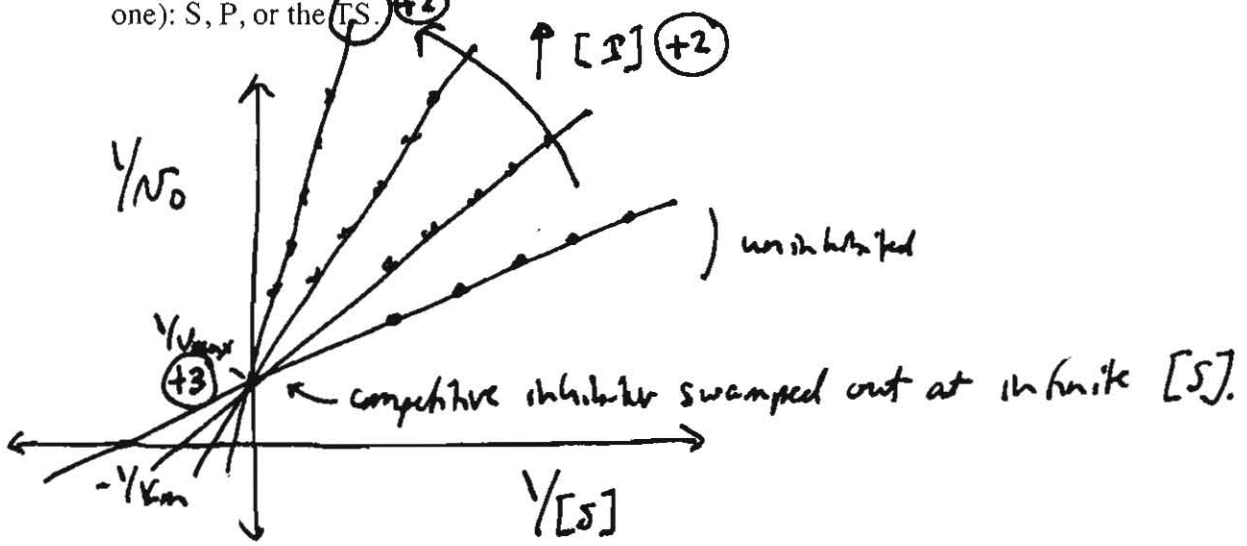
(b; 6 pts) Do you need to know E_T (total enzyme) to determine (circle Y or N for each): K_m (Y/N)? V_{max} (Y/N)? k_{cat} (Y/N)? Why do the estimated values for k_{cat} frequently increase as an enzyme is studied more intensively?

+3

– As the enzyme is purified more and more, and as conditions are altered that maximize its activity (ionic strength, pH, etc.), the apparent measured E_T needed to provide a given activity decreases, so $k_{cat} = V_{max}/E_T$ increases.

(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.

+2



+3 for a Lineweaver-Burke plot

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?

$$v_0 = \frac{k_{cat}[E_T][S]}{[S] + K_m}$$

(+2)

At $[S] \ll K_m$ (operational definition of "low substrate")

$$v_0 = \frac{k_{cat}}{K_m}[E_T][S]$$ (+3) → looks like 2nd-order rate of rxn between E and S.

max achievable value is the diffusion-limited (+3) rate constant

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.

TPP (+2)

(+2) for either
- Functions to provide an electron sink for decarboxylation of α-keto carboxylic acids
- Or as a stabilized carbanion/nucleophile

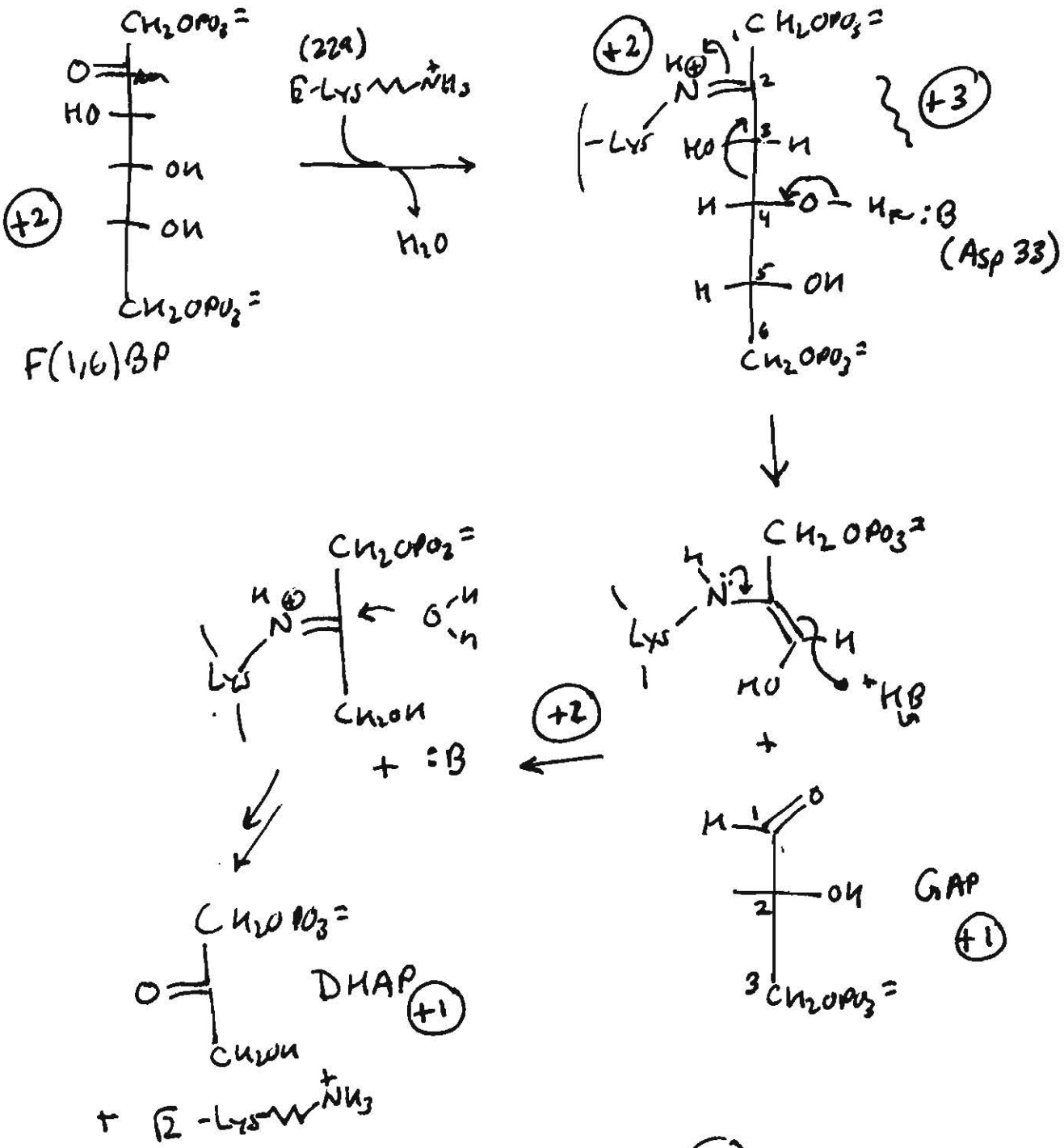
(+2) - M^{++} provides high ⊕ charge density for binding/stabilizing anions like CO_2^- or (P).

or

M^{++} = redox cofactor ∈ ±1 → Zn^{++} and Mg^{++} are not redox active

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?



The Schiff's base serves as an electron sink so that the mech. goes through a stable enamine rather than an unstable enolate - covalent catalysis.

Score for the page_____

(c; 15 pts) Write down the reaction catalyzed by pyruvate carboxylate 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.

(+4) pyruvate + CO_2 + ATP ⟶ oxaloacetate + ADP + P_i

+2 biotin

+3 Pyr. carboxylase is allosterically activated by Acetyl-CoA because OAA is needed to react w/ Acetyl-CoA to bring it into the TCA cycle = feed-forward.

Also adequate Acetyl-CoA means that there is enough energy available in the cell to support gluconeogenesis.

(+3) PEPCK = phosphoenolpyruvate carboxykinase

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.

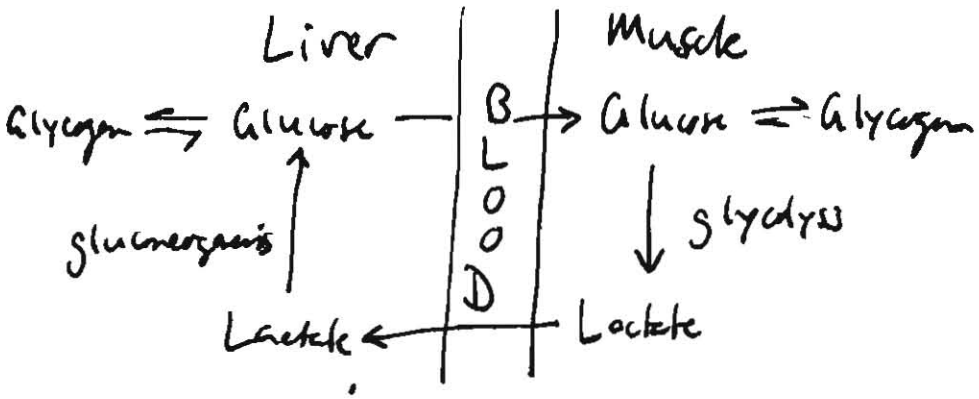
pyruvate + NADH ⇌ lactate + NAD^+ (+2)

this reaction is needed to replenish NAD^+ under anaerobic conditions (+2). When NADH can be oxidized by TCA, then lactate can be re-oxidized, or lactate goes into the Cori cycle.

(+2) The NADH comes from GAP $\xrightarrow{GAPDH}$ 1,3-BPG.

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?



+6

+3 - Liver exports glucose to maintain glucose homeostasis in the blood. Muscle does not - if there's Glc it keeps it and makes glycogen

+3 - Since muscles don't export Glc or do gluconeogenesis, there's no reason they need to listen to "I'm hungry."

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

+4 Liver's job is to maintain ~5mM blood glucose - it will not import glucose unless [Glc] is high. Muscle's job in part is to grab circulating glucose in preparation for activity.

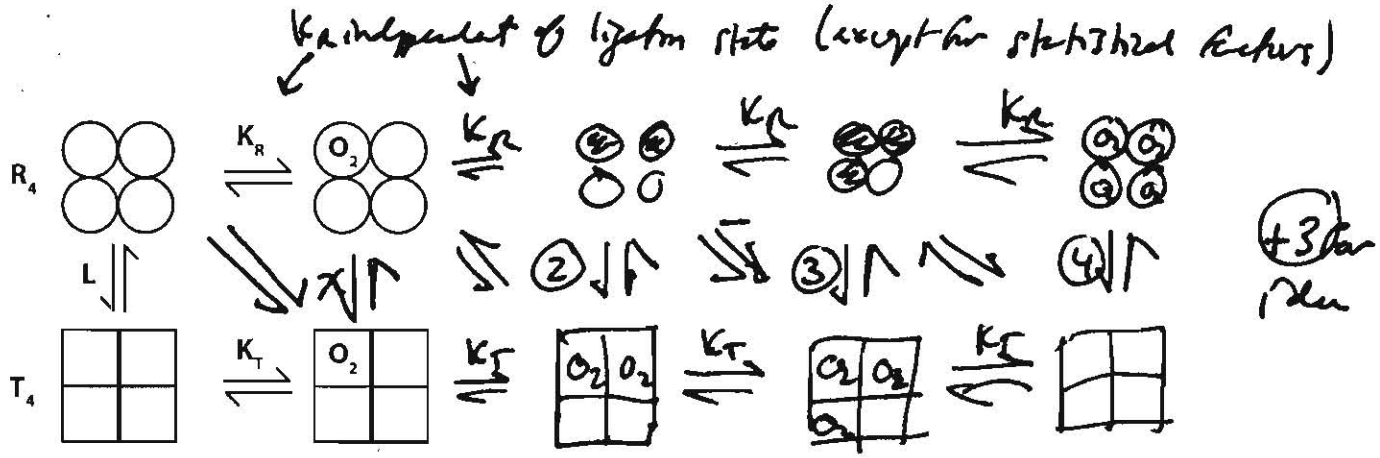
(d; 5 pts) Glucose -> G6P fits at least one criterion for being a useful regulated step. Name the criterion, and give one reason this step isn't highly regulated.

+3 - It's highly exergonic, therefore flux is readily controlled

+2 - But G6P is a branch point - glycogen → G6P so regulating HK does not control flux through glycolysis. Want to regulate a committed step.

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.



(+3) $K_R x = L K_T$

$x = L \frac{K_T}{K_R} << L$ because $K_T << K_R$ (K's are binding constants)

Similarly make more cycles! (+3)

K_{eq} (2) $= L\left(\frac{K_T}{K_R}\right)^2$

K_{eq} (3) $= L\left(\quad\right)^3$

K_{eq} (4) $= L\left(\frac{K_T}{K_R}\right)^4 << L$ (+3)

(ignoring statistical factors)

stronger + stronger preference for R_4 state

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

Score for the page_____