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.

\[
\frac{d[S]}{dt} = 0 \quad E_T = [E] + [ES]
\]
(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?

- As the enzyme is purified more and more, and as conditions are altered that maximize its activity (i.e., strength, pH, etc.), the apparent measured $E_T$ needed to provide a given activity decreases, so $k_{cat} = \frac{V_{max}}{E_T}$ 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 $I_S$.
(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?

$$N_0 = \frac{k_{cat} [E_r] [S]}{[S] + K_m}$$

$${\text{(2)}} \quad \text{At} \quad [S] \ll K_m \quad \text{(operational definition of "low substrate")}$$

$$N_0 = \frac{k_{cat} [E_r] [S]}{K_m} \quad {\text{(3)}} \quad \text{looks like 2nd-order rate of reaction when } [S] \ll K_m.$$ 

max achievable value is the diffusion-limited 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$^{2+}$ or Mg$^{2+}$ in active sites.

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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 to keep the reaction going through a stable enamine rather than an unstable enolate-covalent catalysis.

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.

\[
\text{pyruvate} + \text{CO}_2 + \text{ATP} \rightarrow \text{oxaloacetate} + \text{ADP} + \text{Pi}
\]

+2 biokinetic
+3 pyruvate carboxylase is activated by acetyl-CoA because OAA is needed to react with acetyl-CoA to bring it into TCA cycle = feed-forward.

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

PDK = 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\(^{+}\)

This reaction is needed to replenish NAD\(^{+}\) under anaerobic conditions. When NADH can be oxidized by TCA, then lactate cannot be re-oxidized, so lactate goes into the Cori cycle.

\[\text{NADH comes from GAP} \rightarrow 1,3-BPG\]

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?

Liver

\begin{align*}
\text{Glycogen} & \rightarrow \text{Glucose} \rightarrow \text{Blood} \rightarrow \text{Glucagon} \rightarrow \text{Glycogen} \\
\text{Lactate} & \rightarrow \text{Blood} \rightarrow \text{Lactate}
\end{align*}

Muscle

\begin{align*}
\text{Glucokinase} & \rightarrow \text{G6P} \rightarrow \text{Gluconeogenesis}
\end{align*}

+6

- Liver exports glucon to maintain glucose homeostasis in blood. Muscle does not - it either uses Glc or keeps it and makes glycogen.

+3

- Since muscles don't expect 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 small blood glucose - it will not import glucose unless EGF is high. Muscle's job 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 everywhere, hence flux is readily

+2

- But G6P is a branch point - glycogen -> G6P is key in regulating the direction of flux through glycolysis. Want to regulate a committed step.
(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.

\[ K_R x = L K_T \]
\[ x = L \frac{K_T}{K_R} \ll L \text{ because } K_T \ll K_R \text{ (K's are tiny constants)} \]

Similarly make more cycles! \( \text{(3)} \)

\[ K_{eq \ 2} = L \left( \frac{K_T}{K_R} \right)^2 \]
\[ K_{eq \ 3} = L \left( \frac{K_T}{K_R} \right)^3 \]
\[ K_{eq \ 4} = L \left( \frac{K_T}{K_R} \right)^4 \ll L \text{ (ignoring weak real effects)} \]