Some important stuff to read while you wait –

- The exam has a total of 75 points.
- You are allowed whatever you feel like writing with.
- You can even use a calculator
- You are allowed to use your favorite caffeinated beverage (or any beverage for that matter…)
- For many of these problems I’ve left more white space than you’ll probably need.
- SHOW ALL OF YOUR WORK!
- I know you haven’t slept a wink last night, but DON’T PANIC
- You know more than you think you do, so write it when it comes to mind
- Have a scrumdillyicious summer
1. The following is the catalytic cycle of pyruvate decarboxylase, a thiamine dependent decarboxylase that we discussed in class. Some recent work has been published on this system, increasing the overall level of joy we’ll have with the system. This problem is similar to one last year, so don’t panic.

a. (6 pts) OK, this is a bit long, but shouldn’t be hard since I’ve given you all of the key intermediates. Show the full arrow-pushing mechanism for this transformation. Remember that the base involved in the first step is part of $R_1$, but you can just use a generic $B$: to simplify things.
b. (4 pts) C-2 of pyruvate (the central carbon) was shown to exhibit a primary $^{12}\text{C}/^{13}\text{C}$ kinetic isotope effect (it was measured to be 1.0045\(^1\) (yes, that is primary for carbon)). Using this critical piece of data postulate which step is rate determining and explain, in detail, why (there is more than one possible correct answer). Please ignore all of the extra space; it is to make the next page easier to write.

\[ \text{Reference: Transition-state responses to amino acid perturbations in yeast pyruvate decarboxylase: a carbon kinetic isotope effect study} \]

2. (10 pts) Uncompetitive inhibition is an interesting kinetic model of inhibition, where the inhibitor can only bind to the enzyme-substrate complex. The good news is that this results in a kinetically fairly simple system. Derive the rate law for it. When you are done, indicate what the apparent $K_M$ and $k_{cat}$ are.

$$E + S \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} E \cdot S \overset{k_3}{\rightarrow} E + P$$

$$E \cdot I$$
3. It just wouldn’t be a mechanistic enzymology final exam without a catalytic triad question.
   a. (5 pts) Acetylcholinesterase is an enzyme which catalyses the hydrolysis of acetylcholine at nerve synapses. Propose a mechanism where a catalytic triad, shown below, performs this reaction.

\[
\text{Acetylcholine} \quad \rightarrow \quad \text{Choline}
\]
b. (5 pts) Nerve gas sarin acts on this enzyme. Suggest a mechanism of action, and explain it mechanistically

\[
\begin{align*}
\text{O} & \\
\text{N} & \text{F} \\
\text{O} & \text{O}
\end{align*}
\]
4. Oh, I do like enzyme kinetics! Answer the following questions about a hypothetical enzyme system that was assayed both in the absence and presence of an inhibitor.

<table>
<thead>
<tr>
<th>[S] (µM)</th>
<th>Rate without inhibitor (µmol/min)</th>
<th>Rate with inhibitor at 220 µM (µmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>28</td>
<td>17</td>
</tr>
<tr>
<td>150</td>
<td>36</td>
<td>23</td>
</tr>
<tr>
<td>200</td>
<td>43</td>
<td>29</td>
</tr>
<tr>
<td>500</td>
<td>65</td>
<td>50</td>
</tr>
<tr>
<td>750</td>
<td>74</td>
<td>61</td>
</tr>
</tbody>
</table>

a. (3 pts) Graph the data given above. Be careful to plot the data logically.
b. (3 pts) Calculate the $K_M$ and $v_{max}$ using the graphical data obtained in the absence of inhibitor.

c. (3 pts) Calculate the apparent $K_M$ and $v_{max}$ using the graphical data obtained in the presence of inhibitor.

d. (3 pts) Is the inhibition competitive or noncompetitive? Are you guessing?

e. (3 pts) Calculate $K_I$
5. (8 pts) The β-ketoacyl reductase involved in fatty acid biosynthesis catalyses the reduction of the β-ketone on the nascent fatty acid chain as shown below. Some interesting facts about this enzyme are that the pro-R hydrogen of NADPH shows a kinetic isotope effect of $1.15 \pm 0.02$, and the pro-S hydrogen shows a kinetic isotope effect of $2.6 \pm 0.04^2$. Propose a full arrow pushing mechanism that accounts for the given information.

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6. (8 pts) Dehydroquinate dehydratase catalyzes the conversion of 3-dehydroquinate to 3-dehydroshikimate as shown below. The active site residues involved in this reaction are a non-specified base, and a lysine that is used to form a Schiff base. You may recall the necessity of a twisted boat conformation to have this reaction work, and that the indicated proton is abstracted.
7. (8 pts) Propose a reasonable mechanism for the conversion of phenylalanine to cinnamic acid using the MIO cofactor of a phenylalanine ammonium lyase. Aw, what the heck. It was on last year’s exam so you’ll have practiced it. Think of it as a nice springtime present, like daffodils.
8. (6 pts) The conversion of geranyl pyrophosphate into bornyl pyrophosphate is catalyzed by bornyl pyrophosphate synthetase, a typical terpene cyclase. Write a mechanism for the enzymatic reaction.

Have a great summer everybody. Have a fun time at the end of the year parties, and try to give your brains a bit of a rest somewhere during the sunny months.

Cheers,

Paul