** This examination is open book, <u>but is to be worked on *independently*</u>. You may not discuss or otherwise communicate *any* aspect of the exam with *anyone* other than C. Martin. This includes any discussions with anyone after you are done with the exam, but before the exam's due date and time. This is *very important*.

Name:

Due in LGRT 403D: 4:00pm, Friday, May 24

Show your work for full credit. Be concise, but complete. Avoid long rambling answers which indicate that you don't really understand the question.

1. (25 points) Match the kinetic traces at right with the kinetic mechanisms on the left. For each assignment, **explain in one sentence** the basis for your assignment. You may use Extend to confirm your assignment, but not as an explanation. If there is no match, say so and explain.



Answers:

B builds up quickly, but must build to high levels,. P is delayed. A lag is observed.

ii) __C___

B builds up slowly, but doesn't need to reach high levels. P is delayed. A lag is observed.

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iii) __A___
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No lag in kinetics, therefore 1st order. Fast.

iv) __B___

No lag in kinetics, therefore 1st order. Slow.

2. (25 points) Match the kinetic traces at right with the kinetic mechanisms on the left. For each assignment, **explain in one sentence** the basis for your assignment. You may use Extend to confirm your assignment, but not as an explanation. If there is no match, say so and explain.

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Answers:

Whoops. There was an error in preparing this figure, so one trace does not fit any of the equations. Ah well, the error was big enough that you can easily figure it out.

In the following, note that the $t=\infty$ values tell us about equilibrium distributions.

Overall kinetics intermediate. Overall equilibrium favors A and P, not B. About 1/2 P.

ii) _None_

Overall kinetics intermediate. Overall equilibrium favors B. Should see much less P.

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iii) __D___
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Both steps slow. Overall kinetics slow. Both steps unfavorable. Overall equilibrium unfavorable (P is least favored).

iv) _____A____

Both steps fast. Overall kinetics fast. Both steps favorable. Overall equilibrium favorable.

3. (30 points) Consider the following multi-step kinetic mechanism, involving two substrates:

$$E + S_{1} \underbrace{\underset{k_{-1}=10 \text{ s}^{-1}}{\overset{k_{1}=0.001 \text{ M}^{-1} \text{ s}^{-1}}{\overset{k_{2}=0.01 \text{ M}^{-1} \text{ s}^{-1}}}}_{S_{2}} \underbrace{ES_{1}S_{2}}_{k_{-3}=0.01 \text{ s}^{-1}} E + P$$

You carry out a reaction by pre-incubating 5 μ M E and 5 mM S₁ for a long time. Then you **initiate** the reaction by the rapid addition of a relatively small volume of substrate S₂ to a final concentration of 1 mM.

a) In terms of the concentrations E, S_1 , ES_1 , ES_1S_2 , S_2 , and/or P, and the kinetic constants k_1 , k_{-1} , etc. above, what is the velocity of the reaction (of product appearance) at any given time? This is a "quick answer" question.

$$\frac{\partial P}{\partial t} = k_3 [ES_1S_2] - k_{-3}[E][P]$$

b) What is the actual velocity of the reaction at time zero (immediately after addition of $S_{\rm 2})?$

Zero. The concentration of ES_1S_2 will not have had time to build up at all.

c) To estimate the initial velocity of the reaction, what simplifying assumption(s) might you make before trying to derive anything? Why are these assumptions valid (be sure that they are!)?

$$\begin{bmatrix} P \end{bmatrix} \approx 0 \qquad \therefore \qquad \frac{\partial P}{\partial t} \approx k_3 \begin{bmatrix} ES_1 S_2 \end{bmatrix}$$
$$\begin{bmatrix} S_1 \end{bmatrix} \approx \begin{bmatrix} S_1 \end{bmatrix}_T \qquad \begin{bmatrix} S_2 \end{bmatrix} \approx \begin{bmatrix} S_2 \end{bmatrix}_T$$

The first, because early in the reaction, very little product has yet been formed.

The second, because very little substrate has been consumed.

d) What are the starting concentrations of all species when you initiate the reaction by the addition of S_2 ?

$$[E]_{o} = 5 \ \mu M \qquad [ES_{1}]_{o} = 2.5 \ x \ 10^{-12} \ M$$
$$[S_{1}]_{o} = 5 \ m M \qquad [ES_{1}S_{2}]_{o} = 0$$

 $[P]_{o} = 0$

$$K = \frac{k_1}{k_{-1}} = \frac{[ES_1]}{[E][S_1]} \cong \frac{x}{(E_T - x)[S_1]} \qquad [ES_1] = 2.49999875x10^{-12}M \neq 0$$

$$[ES_1] = x = \frac{K[E_T][S_1]}{1 + K[S_1]} = \frac{[E_T]}{\frac{k_{-1}}{k_1}\frac{1}{[S_1]} + 1} \qquad [S_2] = [S_2]_T$$

$$[E] = 4.9999975x10^{-6}M \approx [E]_T$$

Note that the number of significant figures above is inappropriate for any "real" experiment. They are presented for your information only...

e) Develop the full set of differential equations describing the kinetics accompanying this rapid mix experiment. Use $Extend^{TM}$ to plot the kinetics for the time evolution of the various species.

$$\begin{aligned} \frac{d[E]}{dt} &= -k_1[E][S_1] + k_{-1}[ES_1] + k_3[ES_1S_1] - k_{-3}[E][P] \\ \frac{d[S_1]}{dt} &= -k_1[E][S_1] + k_{-1}[ES_1] \\ \frac{d[ES_1]}{dt} &= k_1[E][S_1] - k_{-1}[ES_1] - k_2[ES_1][S_2] + k_{-2}[ES_1S_2] \\ \frac{d[ES_1S_2]}{dt} &= k_2[ES_1][S_2] - k_{-2}[ES_1S_2] - k_3[ES_1S_1] + k_{-3}[E][P] \\ \frac{d[S_2]}{dt} &= -k_2[ES_1][S_2] + k_{-2}[ES_1S_2] \\ \frac{d[P]}{dt} &= k_3[ES_1S_1] - k_{-3}[E][P] \end{aligned}$$

The pictorial form of the above is:



We'll leave the plotting to you. Yes the parameters make for very little product accumulation!!

4. (20 points) In temperature-jump experiments, one starts with a system at equilibrium. The temperature is then rapidly (instantaneously) increased, such that the system is no longer at equilibrium. With time, the system then reacts ("relaxes") to satisfy the new equilibrium conditions.

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You are studying the unfolding of a protein in a temperature jump experiment. To initiate the unfolding you jump the temperature from 35° C to 55° C very rapidly (your instrument can uniformly raise the temperature of the sample 2000° s⁻¹). Following the CD signal for the protein, you get the following plot of apparent percent folded protein (100 corresponds to the CD signal for fully folded protein; 0 for fully unfolded protein).



Your colleague looks at the above data and proclaims "this is not a simple twostate unfolding reaction!" She is right – explain (you need not derive elaborate exact equations). What might you conclude about the properties of the intermediate state(s)? Feel free to use simulations to bolster your conclusions.

The **initial lag** tells us that this is not the simple exponential decay we expect from a twostate model. The real story must be more complex.

In fact, the data were collected from a system following: $F \xleftarrow{2}{0.2} I \xleftarrow{5}{0.2} U$

One can conclude that the **intermediate must have spectral properties similar to those of the folded (F) form** (ie. $F_{app}=F+I$. If the properties were more those of the unfolded form, the time dependence of F_{app} would look more like the trace for F alone.

Things one cannot conclude:

Pre-equilibrium. Look at the rate constants above. No pre-equilibrium there. That it first follows 2^{nd} order kinetics and then 1^{st} order. It's 2^{nd} order throughout. Anything about ΔH , ΔS , ΔCp , or other thermodynamic properties (other than K/ ΔG). A more complete plot is shown below, demonstrating the time dependence of the concentrations of both F and I.

