Exam #4 Chem 728 Spring 2002 Name: ________________

** 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***.

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.

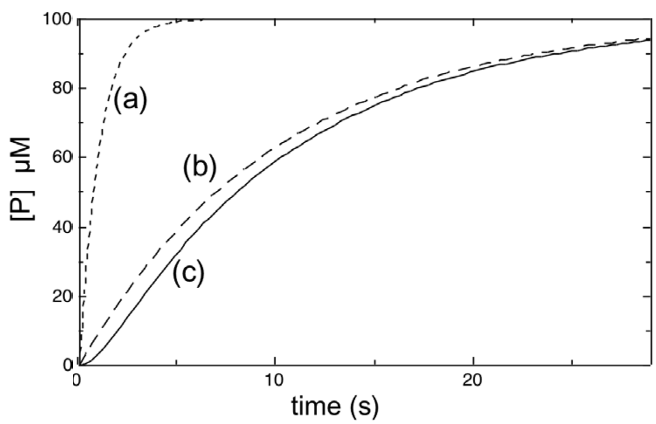
1. (25 points) Match the kinetic traces at right with the kinetic mechanisms on the left. For each assignment, **explain in one sentence** the basis for your assignment. You may use Extend to confirm your assignment, but not as an explanation. If there is no match, say so and explain.

i) $A \xrightarrow{1.0\ s^{-1}} B \xrightarrow{0.1\ s^{-1}} P$

ii) $A \xrightarrow{0.1\ s^{-1}} B \xrightarrow{1.0\ s^{-1}} P$

iii) $A \xrightarrow{1.0\ s^{-1}} P$

iv) $A \xrightarrow{0.1\ s^{-1}} P$



Answers:

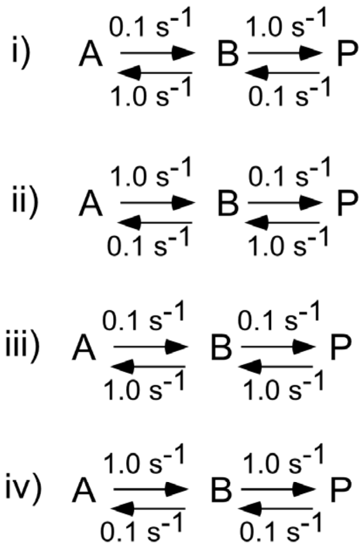
i) ______

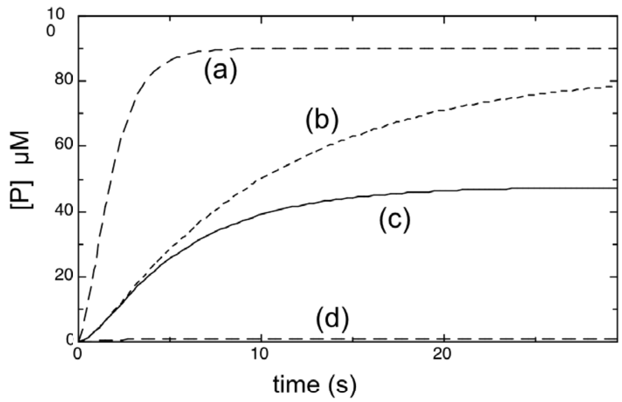
ii) ______

iii) ______

iv) ______

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.





Answers:

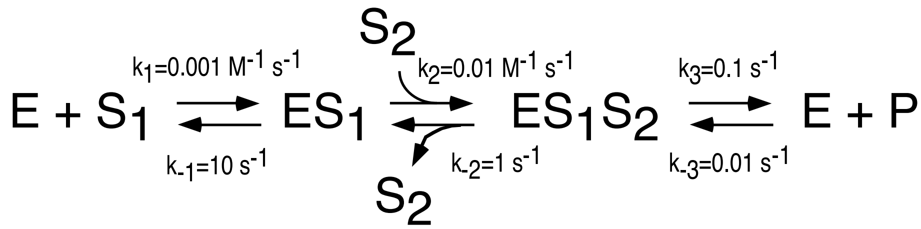
i) ______

ii) ______

iii) ______

iv) ______

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



You carry out a reaction by pre-incubating 5 μM E and 5 mM S_1 for a long time. Then you **initiate** the reaction by the rapid addition of a relatively small volume of substrate S_2 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.

b) What is the actual velocity of the reaction at time zero (immediately after addition of S_2)?

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!)?

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

$[E]_o$ =

$[ES_1]_o$ =

$[S_1]_o$ =

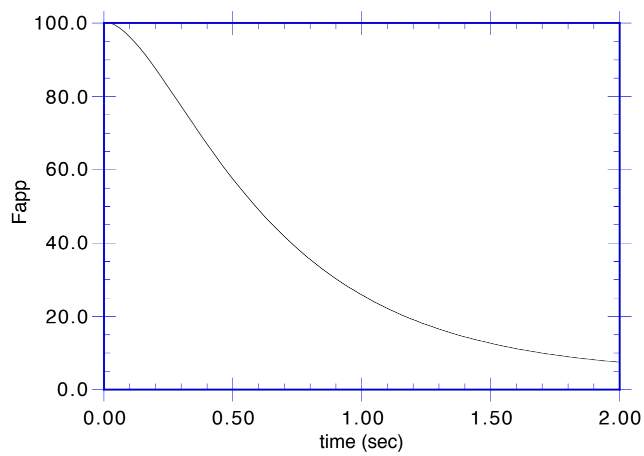
$[ES_1S_2]_o$ =

$[P]_o$ =

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

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.

   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 two-state 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.