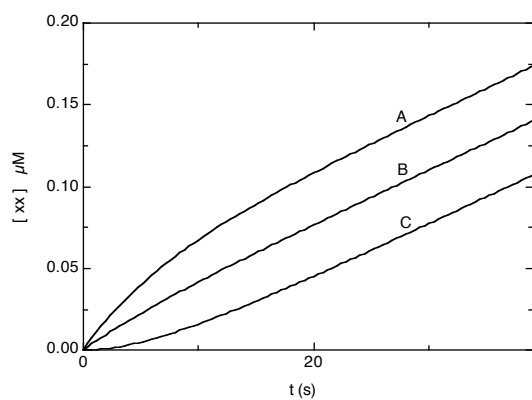** This examination is open book, <u>but is to be worked on **_independently_**</u>. You may not discuss or otherwise communicate *any* aspect of the exam with *anyone* other than C. Martin. This includes any discussions with anyone after you are done with the exam, but before the exam's due date and time. This is **_very important_**.
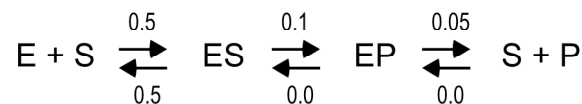
Due in LGRT 403D, 9:30am, Monday, May 19

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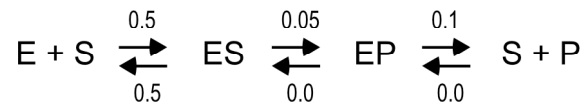*Avoid long rambling answers which indicate that you don't really understand the question.*

1. (25 points) Consider the following experimental kinetic traces:

Kinetic Mechanism #1

$$E + S \underset{0.5}{\overset{0.5}{\rightleftharpoons}} ES \underset{0.0}{\overset{0.1}{\rightleftharpoons}} EP \underset{0.0}{\overset{0.05}{\rightleftharpoons}} S + P$$

Kinetic Mechanism #2

$$E + S \underset{0.5}{\overset{0.5}{\rightleftharpoons}} ES \underset{0.0}{\overset{0.05}{\rightleftharpoons}} EP \underset{0.0}{\overset{0.1}{\rightleftharpoons}} S + P$$

In each case, $[E]_{Tot} = 0.1 \; \mu M$.

Rate constants are either s^{-1} or $\mu M^{-1} s^{-1}$, as appropriate.

a) (10 points) There are actually FOUR traces in the kinetic traces above, but two are indistinguishable at this level. Indicate with a letter, the trace which corresponds to each of the following

_____ Kinetic Mechanism #1, follow [P] only
_____ Kinetic Mechanism #1, follow [P]+[EP]
_____ Kinetic Mechanism #2, follow [P] only
_____ Kinetic Mechanism #2, follow [P]+[EP]

b) (5 points) Explain briefly why they all reach the same steady state velocity.

c) (5 points) Which trace(s) show(s) burst phase kinetics

d) (5 points) Which trace(s) show(s) lag phase kinetics?

2. (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 (because $\Delta G/K$ depends on temperature). 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).
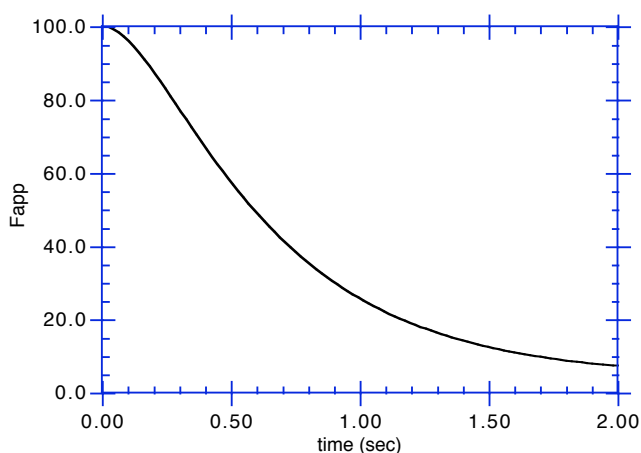

Your colleague looks at the above data and proclaims "this is not a simple two-state unfolding reaction!"

a) (10 points) She is right – explain (you need not derive elaborate exact equations).

b) (10 points) What might you conclude about the properties of the intermediate state(s)?

Feel free to use simulations to bolster your conclusions.

3. (25 points) The data at right show the titration of a ligand (L) on to a protein (P). The protein contains a single binding site for the ligand and is present at a concentration of 10!nM. The proteins (and sites) behave independently, as do the ligands, and you can assume that the volume doesn't change during the titration. Explain the data and estimate the equilibrium dissociation constant (K_d).
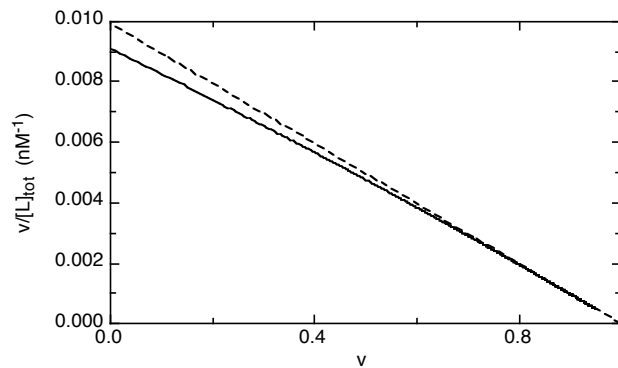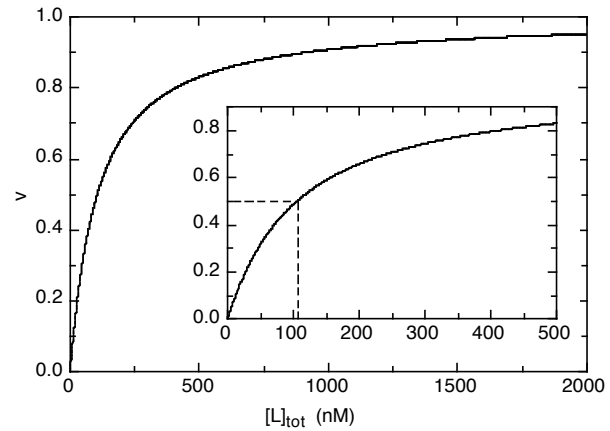
4. (30 points) The data at right show the titration of a ligand (L) on to a protein (P). The protein contains a single binding site for the ligand and is present at a concentration of 10!nM. The proteins (and sites) behave independently, as do the ligands, and you can assume that the volume doesn't change during the titration. Explain the data and estimate the equilibrium dissociation constant (K_d). You will need to derive an equation for this purpose – show your work (please derive it on scratch paper, and then transfer the cleaned derivation to the exam).