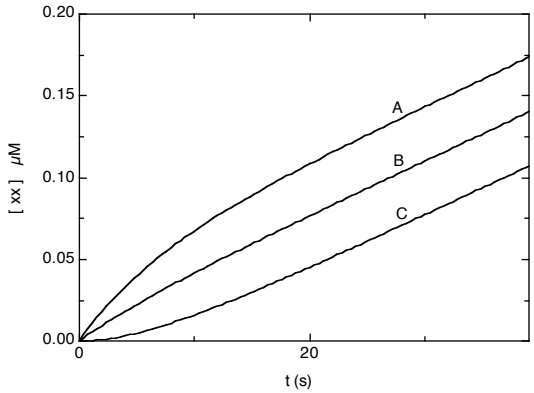
** This examination is open book, but is to be worked on ***independently***. You may not discuss or otherwise communicate *any* aspect of the exam with *anyone* other than C. Martin. This includes any discussions with anyone after you are done with the exam, but before the exam's due date and time.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

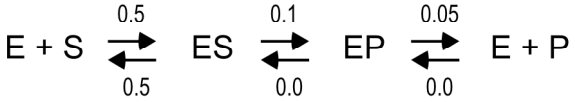
Due in LGRT 403D, 9:30am, Monday, May 19

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

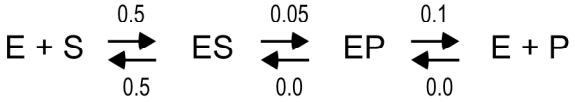
1. (25 points) Consider the following experimental kinetic traces:



Kinetic Mechanism #1



Kinetic Mechanism #2



In each case, $[E]_{Tot}$ = 0.1 μM.

Rate constants are either s^{-1} or μM^{-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

C __ Kinetic Mechanism #1, follow [P] only
A __ Kinetic Mechanism #1, follow [P]+[EP]
C __ Kinetic Mechanism #2, follow [P] only
B __ Kinetic Mechanism #2, follow [P]+[EP]

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

Simply, in each case, the rate determining step has a first order rate constant of 0.05 (3rd step in Mech #1; 2nd step in Mech #2). At steady state, the corresponding intermediate just prior to the rate determining step builds up to [E]tot levels. The velocity is then that level (0.1 μM) times 0.05, the steady state slopes above.
The fact that they both have the same rates for E+S binding and dissociation is also a factor, as that impacts the steady state level of the intermediate. Partial credit for that.

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

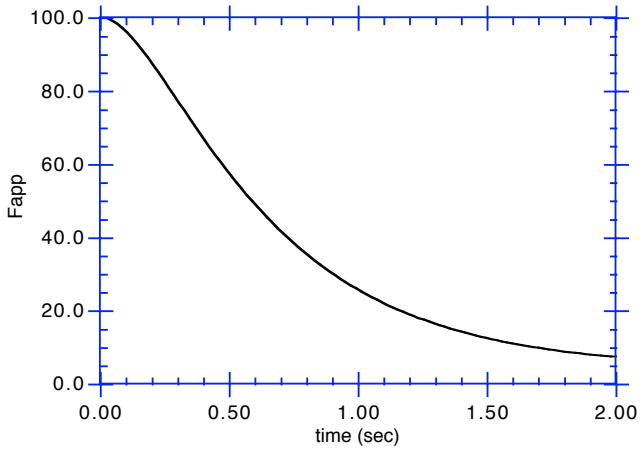
A

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

C

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 ΔG/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°C 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).

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

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

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

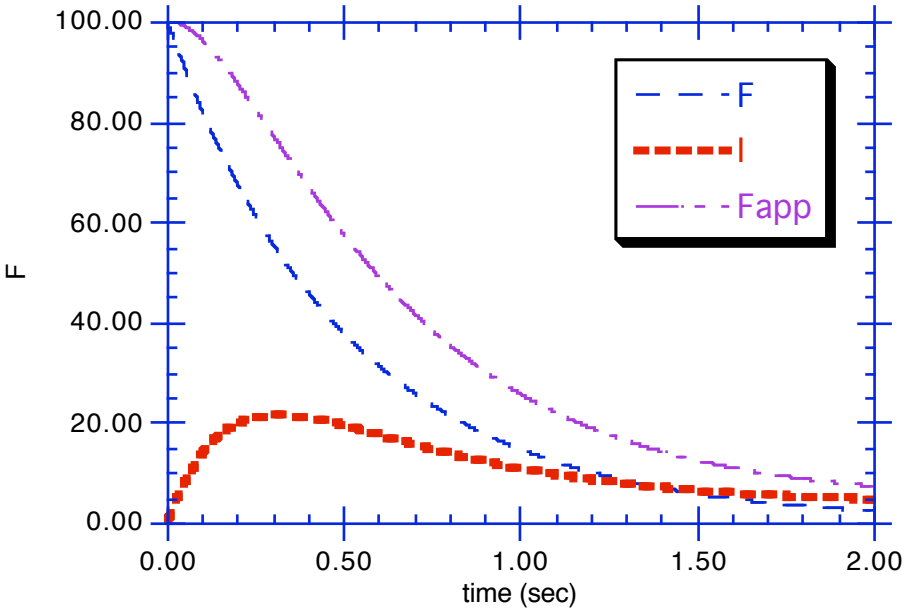
Feel free to use simulations to bolster your conclusions.

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.

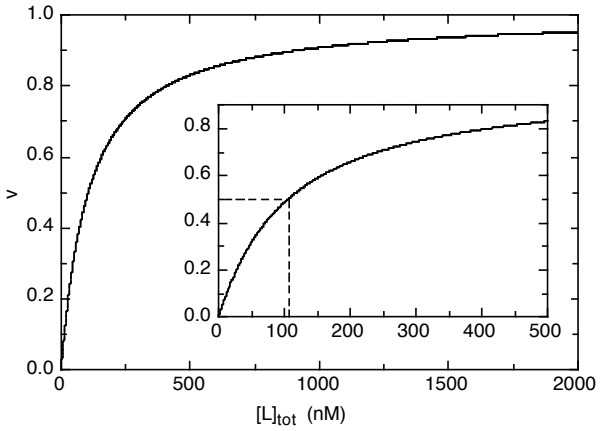
Things one **cannot** conclude:
Pre-equilibrium. Look at the rate constants above. No pre-equilibrium there.
That it first follows 2nd order kinetics and then 1st order. It's 2nd order throughout.

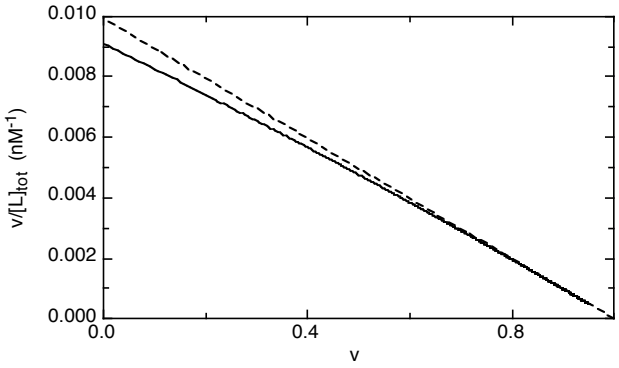
Anything about ΔH, ΔS, ΔCp, or other thermodynamic properties (other than K/ΔG).

A more complete plot is shown below, demonstrating the time dependence of the concentrations of both F and I.



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).





This is an easy question. You can get Kd from either plot.

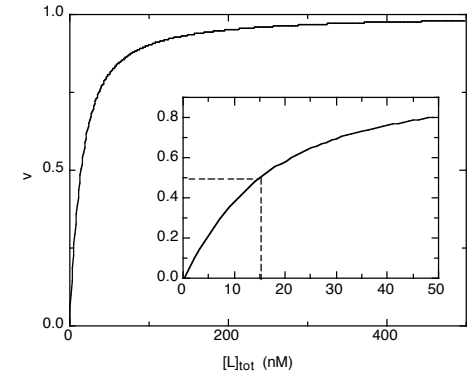
From the top plot, remember that the concentration at which binding is half-saturated is K_d (given assumptions discussed in question 2). Using the inset, we obtain K_d = 110 nM

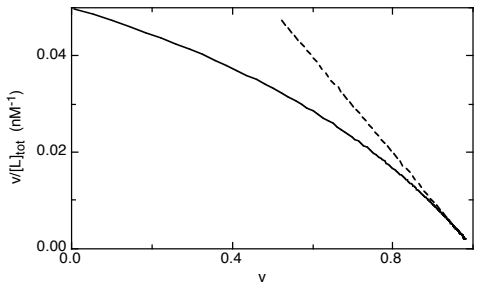
From the lower plot, we can use the Scatchard equation

$$\frac{\theta}{[L]} = K_a(1-\theta)$$

to obtain K_a from either the y–intercept (0.009 nM^{-1}), yielding K_d = 111 nM (or perhaps 100 nM, what do you think?). Or from the slope (you figure it out).

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).





K_d = 10 nM

We start off as before, with the three equations.

$$[P]_{tot} = [P] + [PL] \qquad [L]_{tot} = [L] + [PL] \qquad K_a = \frac{1}{K_d} = \frac{[PL]}{[P][L]}$$

Scatchard analysis makes the approximation $[PL] << [L]$ so that $[L]_{tot} \approx [L]$

We can't make that assumption, so $\frac{1}{K_d} = \frac{[PL]}{([P]_{tot} - [PL])([L]_{tot} - [PL])}$

Multiplying out yields:

$$([P]_{tot} - [PL])([L]_{tot} - [PL]) - K_d[PL] = 0$$
$$[PL]^2 - ([P]_{tot} + [L]_{tot} + K_d)[PL] + [P]_{tot}[L]_{tot} = 0$$

$$[PL] = \frac{([P]_{tot} + [L]_{tot} + K_d) - \sqrt{([P]_{tot} + [L]_{tot} + K_d)^2 - 4[P]_{tot}[L]_{tot}}}{2}$$ this was derived in class.

Now, find the concentration of $[L]_{tot}$ at which $[PL] = 0.5\,[P]_{tot}$

$$\frac{[P]_{tot}}{2} = \frac{([P]_{tot} + [L]_{tot} + K_d) - \sqrt{([P]_{tot} + [L]_{tot} + K_d)^2 - 4[P]_{tot}[L]_{tot}}}{2}$$

$$0 = [L]_{tot} + K_d - \sqrt{([P]_{tot} + [L]_{tot} + K_d)^2 - 4[P]_{tot}[L]_{tot}}$$

$$([L]_{tot} + K_d)^2 = ([P]_{tot} + [L]_{tot} + K_d)^2 - 4[P]_{tot}[L]_{tot}$$

$$[L]_{tot}^2 + 2[L]_{tot}K_d + K_d^2 = [P]_{tot}^2 + [L]_{tot}^2 + K_d^2 + 2[P]_{tot}[L]_{tot} + 2[P]_{tot}K_d + 2[L]_{tot}K_d - 4[P]_{tot}[L]_{tot}$$

$$0 = [P]_{tot}^2 + 2[P]_{tot}[L]_{tot} + 2[P]_{tot}K_d - 4[P]_{tot}[L]_{tot} = [P]_{tot} - 2[L]_{tot} + 2K_d$$

$$[L]_{tot} = \frac{[P]_{tot} + 2K_d}{2} = K_d + \frac{1}{2}[P]_{tot} \qquad K_d = [L]_{tot} - \frac{1}{2}[P]_{tot}$$

Remember that [L]tot here is the concentration of [L] at which the protein is half-saturated.

From the upper plot, we have $K_d = [L]_{tot} \Box \frac{1}{2}[P]_{tot} = (15nM) \Box \frac{1}{2}(10nM) = 10nM$

A simpler derivation that some of you came up with (and which is perfectly acceptable) notes that the equations leading to the conclusion "K_d is the concentration of L at which saturation is half maximal" ARE valid IF you consider L to be the ***free*** ligand concentration (not total).

Hence, at half saturation, $[L]_{tot}$ = 15 nM, but $[PL] = 0.5[P]_{tot}$ = 5 nM, therefore $[L]_{free}$ = 10 nM.