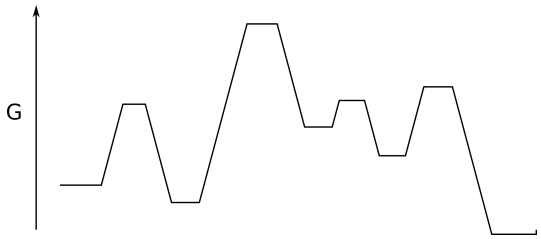
Instructions:

- Turn in your results in some kind of clear form (handwritten, typed, pdf).
- Feel free to work in groups.
- I have tried to be consistent in my language in my prompts if I want something specific.
  - *Sketch:* Hand draw a plot. Axes should be labeled conceptually, with key features indicated and explained.
  - *Generate a plot:* Plot using software. Label axes, use appropriate significant figures, etc.
  - *Calculate:* Actually calculate numbers using math. Report units and uncertainty, as appropriate.
  - *Describe/Argue:* Use any combination of writing, sketching, plotting, and calculation to argue for an interpretation.
  - Finally, I will sometimes specify the sort of explanation I want.
    - *Molecular*: Describe what the atoms and molecules are doing in space and time. Depending on the context of the question, this might also involve explaining the result in terms of atomic properties like hydrophobicity.
    - *Energetic*: Describe in energetic terms (entropy, free energy, statistics).
    - *Mathematical*: Answer in terms of how the functions behave. For example, if I asked "mathematically, why does $Kx/(1+Kx)$ saturate with increasing x" the answer would be: "because as $x \to \infty$, $Kx \gg 1$ and the function tends to 1."
- Some of the questions may require you to play with math and/or ideas we did not explicitly discuss in class. This is intentional. Many times in science you will be faced with a paper that uses an approach you are not familiar with. Learning how to gather enough information to critically evaluate their findings, as well as understand any mathematical models employed, is important.
- Some questions are listed as **GRAD STUDENT** questions. Undergrads are free to do those questions, but it is not required.

6 Kinetics

1. The energy diagram for a multi-intermediate enzyme mechanism is shown below:



   (a) You determine that a mutation of the enzyme causes a 250-fold decrease in k_{obs} (rate of product accumulation). What is the change in the energy of the transition state for the mutant compared to the wt enzyme at 300 K?

   (b) Can you determine which elementary step(s) have been affected by the mutation based on your experiments? Why or why not?

2. Consider the following reaction:

$$A \rightarrow^{k_1} B \rightleftarrows_{k_3}^{k_2} C$$

   (a) Write out a transition matrix for the reaction.

   (b) If you start with the concentrations $[A_0, B_0, C_0]$, what is the concentration of $[B]$ after a short time Δt?

3. In some biological reactions, two molecules of A come together, react, and yield A and A' as products:

$$A + A \rightleftarrows_{k_2}^{k_1} A \cdot A \rightarrow^{k_3} A + A'$$

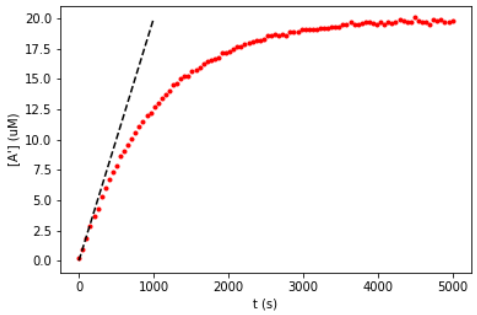
   You are studying such a reaction. You read a paper in which the authors report rate constants for k_1, k_2, and k_3:

$$k_1 = 10\ \mu M^{-1} \cdot s^{-1}$$

$$k_2 = 10\ s^{-1}$$

$$k_3 = 0.001\ s^{-1}$$

   You set out to see if you an reproduce their measurements. You have an assay for following the concentration of A' in solution. You start with [A]=20 μM, $[A'] = 0\ \mu M$ and observe the following curve.



The initial slope (dashed line) is:

$$\frac{d[A']}{dt} = 0.02\ \mu M \cdot s^{-1}$$

Are your results consistent with theirs? Why or why not?

4. You are studying an enzyme that converts a non-fluorescent substrate into a fluorescent product. You use a flourimeter to measure the fluorescence of a buffer sample as $18,989$ units and $100\ \mu M$ product as $289,108$ units. You add substrate at differing concentrations (given in μM) to a solution containing $1\ nM$ enzyme and then collect the data in "observation.csv."

   (a) What is the apparent rate constant under each reaction condition?

   (b) What is k_{cat} and K_M?

   (c) From these data, can you tell if the reaction is reversible?

5. Write out the reaction scheme consistent with this transition matrix.

$$T = \begin{bmatrix} 0.991 & 0.001 & 0.000 & 0.000 \\ 0.001 & 0.999 & 0.010 & 0.000 \\ 0.000 & 0.000 & 0.980 & 0.001 \\ 0.008 & 0.000 & 0.010 & 0.999 \end{bmatrix}$$

6. The fastest enzymes end up being *diffusion limited*. What does this mean, and why can't diffusion limited enzymes go any faster? (Put another way, why doesn't it help to lower the activation energy after some point?).

7. You are studying spontaneous unimolecular reactions at $25\ ^\circ C$.

   (a) The reaction passes through a single transition state whose energy is $100\ kJ \cdot mol^{-1}$ greater than the reactant. What is the fold speed up ($10\times$, $20\times$, etc.) if a catalyst is added that lowers the transition state energy by $25\ kJ \cdot mol^{-1}$?

   (b) Now consider a reaction with a more complicated energy barrier. It passes through two transition states: the first is $100\ kJ \cdot mol^{-1}$ higher in energy than the reactant, the second is $25\ kJ \cdot mol^{-1}$ higher in energy than the reactant. The catalyst stabilizes the first transition state by $25\ kJ \cdot mol^{-1}$ but has no effect on the second transition state. How much will this speed up the reaction?

   (c) Could you tell the difference between the reaction/enzymatic mechanisms on parts a and b from their reaction kinetics? Why or why not?