AS.020.674 – Quantitative Biology and Biophysics; Spring 2022

Exam 4: Kinetics

Instructions:

- Below are 2 sections, each with multiple questions (pages 2 – 5). Submit your answers in a separate document in pdf format.
- Make sure that it is unambiguously clear in your pdf document what question any of your answer/content refers to.
- The exam is **open note and open book**.
- You **may use** any resources provided in class and any resources you find on the internet.
- You **may not** collaborate with others during the exam.
- You **may not** paste diagrams or text that is not your own or copy text verbatim for your answers.
- If a question involves calculations, make sure to clearly outline how you arrived at your result, i.e. explicitly write the equation. Listing just the final results is insufficient.
- Make sure to define **any** variable or parameter you use in your equation, even if their definition may seem obvious. If there is any ambiguity, we may not award full points.
- Keep your answers **brief and concise**.
- You have **24 hours** to complete the exam (starting at 9:30am on Wed, May 4, ending at 9:30am on Thu, May 5).
- Please double-check before submission that your pdf file contains all your answers.
- If you have any problems with understanding the questions or think you found an error, please email Christian (kaiser@jhu.edu), Kevin (kmaciub1@jhu.edu) or Xiuqi (cxiuqi1@jhu.edu).
- You must submit the pdf file with your answer through Blackboard **by 9:30am on Thu, May 5**. Do not wait until the very last minute to submit. In case of technical difficulties with Blackboard, make sure to email your answers to Kevin or Xiuqi.
- **Sign the ethics pledge below** with your name and date it, and **submit the signed pledge** together with your answers.

"I agree to complete this quiz without unauthorized assistance from any person. I will not discuss or share the exam questions, answers or any exam-related material with anyone until 9:30 AM East Coast Time, on Thursday, May 5, 2022."

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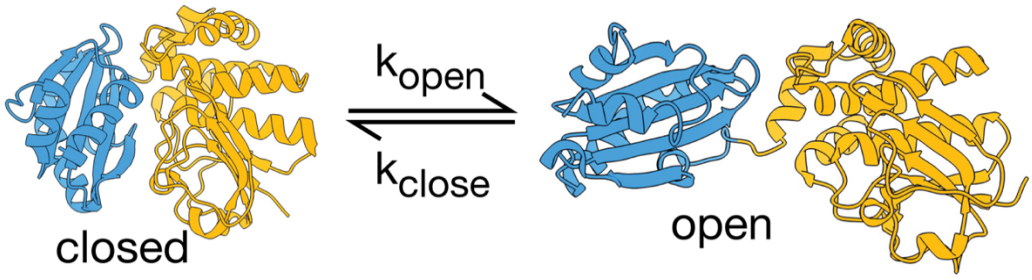
Signature, Date

Good luck with the last exam!

I. Conformational change kinetics (20 points)

You study a protein that undergoes a large conformational change, transitioning between a closed and an open state as depicted in the figure below.



I.1 Do you expect the transition from the closed to the open state to obey zero-order, first-order or second order kinetics? Explain your reasoning. (1 points)

I.2 For the forward and reverse reactions shown above, provide mathematical expressions for the change in concentration of the open and closed states with time, i.e. for d[closed]/dt and d[open]/dt, where [closed] and [open] are the concentrations of the open and closed states. (NB: This question does not ask for specific values, but for expressions.) (2 points)

I.3 If you start the reaction at time t = 0 with all molecules in the closed state (i.e. the initial concentration of the open state is $[open]_0 = 0$), how does [closed] depend on time t and the rate constant(s)? Provide an equation and explain the type of dependence of [closed] on time (linear, quadratic, single-exponential, double-exponential, inverse, ...). (2 points)

I.4 Using the equation you provided in I.3 as a starting point, develop a mathematical expression for the concentration of the open state, [open], as a function of time and rate constant(s). Assume the same reaction conditions as in I.3 (i.e. $[open]_0 = 0$). Make sure to explain how you arrive at the expression based on the equation you provided in I.3. (3 points)

I.5 Assume that the rate of opening is temperature-dependent, with values of $k_{open}(25°C) = 10^4\ s^{-1}$ and $k_{open}(22°C) = 8.4*10^3\ s^{-1}$ at 25°C and 22°C, respectively. If the equilibrium constant is $K_{eq}(25°C) = 0.2$ at 25°C, what is the value of the equilibrium constant at 22°C? Assume that k_{close} does not change significantly within the temperature range of 22 to 25°C. (1 points)

I.6 How many transitions from the open to the closed state occur every second under equilibrium conditions at 25°C if the total protein concentration is [protein] = 5 µM and the reaction volume is $V_{reaction}$ = 100 µl? (Use the values from I.5 for k_{open} and K_{eq}.) (3 points)

I.7 How do the rate constants and the equilibrium constant change if the protein is diluted 10-fold, from 5 µM to 500 nM? Explain your answer. (1 point)

I.8 Tryptophan (Trp) residues can often be exploited as endogenous probes to monitor conformational changes in proteins. If the closed state exhibits higher Trp fluorescence than the open state, does a shift in temperature from 22°C to 25°C result in an increase or a decrease in fluorescence? Explain your reasoning. Assume that the fluorescence properties of Trp are the same at both temperatures and use the kinetic parameters from I.5. (1 point)

I.9 In actual experiments, achieving the starting condition of $[open]_0$ described in I.3 is often not feasible for a conformational transition like the one shown above. Measuring equilibrium constants, on the other hand, is often easier. Let's say that you know what the Trp fluorescence emission intensities are for the closed and for the open state, and that you know from equilibrium measurements what the equilibrium constants are at a variety of conditions (e.g. different concentrations, temperatures, ...), but you have no information about either of the two rates. Suggest an experimental strategy for determining both k_{open} and k_{close}. Be specific as to what you measure, what (if any) parameter(s) you vary, and how many measurements you have to minimally carry out to obtain the two rate constants. Make sure to include any equation(s) needed to relate the two rate constants to your measured quantity. (6 points)

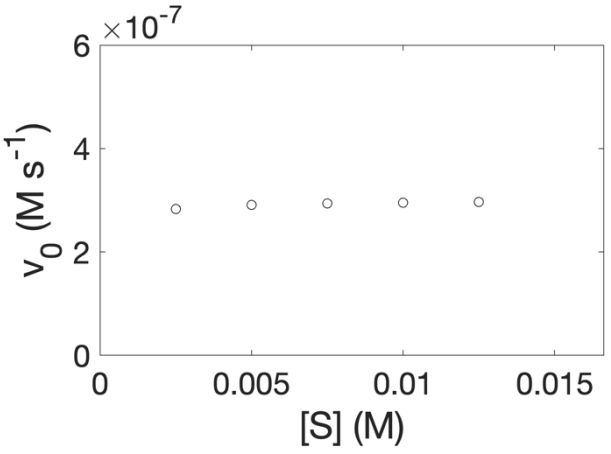
II. Enzyme kinetics (20 points)

II.1 You want to experimentally characterize an enzyme that is well described by Michaelis-Menten kinetics. The enzyme-catalyzed reaction can be described as

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E{\bullet}S \xrightarrow{k_2} E + P$$

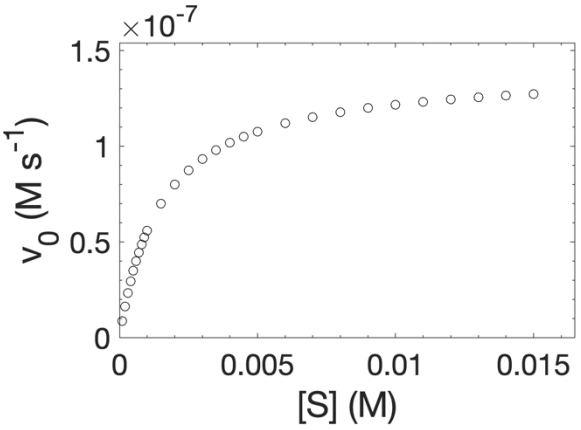
From a comparison with a related enzyme, you estimated that the Michaelis constant, K_m, is approximately 25 mM. Using your own words and the terminology of the Michaelis-Menten model (K_m, maximum velocity V_{max}, initial velocity v_0), explain what K_m represents. (2 points)

II.2 Based on your assumptions, you carry out an experiment. You measure the initial velocities at five different substrate concentrations and obtain the result shown in the figure on the right, which does not look like a typical Michaelis-Menten plot (such as the one shown in II.3).
What could likely account for the discrepancy between the expected and the observed result? Select one or more of the answers below. Explain your reasoning for selecting the answer(s) and describe how you would change your experimental conditions in order to characterize the enzyme. (3 points)



a. the enzyme concentration is too high
b. the enzyme concentration is too low
c. the substrate concentration is too high
d. the substrate concentration is too low
e. the time resolution of the measurement is too low
f. the measurement time is too short.

II.3 Instead of fixing the problem you identified in II.2, you decide to characterize a homolog of the enzyme from a different species and obtain the data shown on the right, using an enzyme concentration of 10 nM. Based on the plot on the right, estimate V_{max}, K_m, k_{cat} and the catalytic efficiency of this enzyme. (Note: You do not need to determine these parameters precisely by fitting – an estimate based on the plot is sufficient, but make sure to explain how you obtained it.) (2 points)



II.4 You obtain a substrate-analog for your enzyme. (A substrate analog here is a small molecule that has a similar chemical structure as the substrate.) The substrate-analog is non-fluorescent when free in solution, but is highly fluorescent when bound to the enzyme. Its binding characteristics are the same as those of the real substrate, but the analog does not react to give the product (i.e., $k_2 = 0$ for the analog). You want to use the substrate-analog to independently measure the substrate binding and dissociation rates (k_1 and k_{-1}) for your enzyme. To this end, you mix the enzyme with the total concentration of analog indicated in the table. By following the change in fluorescence that is caused by the analog binding to the enzyme, you obtain the observed rate constants of binding, k_{obs}, listed in the table. The enzyme concentration is 10 nM in all reactions.

[analog] (mM)	k_{obs} (s^{-1})
0.1	2.0
0.3	4.0
1.0	11.0
3.0	31.0
10.0	101.0

a. Provide mathematical expressions that relate k_{obs} to the rate constants for binding and dissociation (k_1 and k_{-1}).
b. Provide a mathematical expression that relates the fluorescent signal to k_{obs}.
c. Explain the simplifying assumption(s) that are required to obtain these mathematical expressions, and why they are justified.
d. Calculate the values of k_1 and k_{-1} from the data provided in the table.

(8 points total)

(Hint: You do not have to think of the protein as an enzyme in the context of the non-reacting analog. Think of it as a protein binding a ligand.)

II.5 Would you expect the substrate-analog described in II.4 to act as an inhibitor in reactions containing the enzyme and the substrate? Choose one answer and explain your reasoning. (2 points).

The substrate analog

a. acts as a competitive inhibitor
b. acts as a reversible non-competitive inhibitor
c. acts as a substrate-dependent non-competitive inhibitor
d. dos not act as an inhibitor

II.6 A similar enzyme to the one that you are studying catalyzes a reaction in a metabolic pathway. Increasing the overall rate of substrate-to-product conversion would confer a fitness advantage to the cell. Let's say that you can introduce a change into the enzyme that has one of the following consequences:

a. increase k_1 by a factor of 2
b. decrease k_{-1} by a factor of 2
c. increase k_2 by a factor of 2

Which of these changes would be expected to have the largest positive impact on cellular fitness? Choose one answer and why one of the changes has a larger impact than the others under these conditions. (3 points)

The substrate concentration in the cell is 5 mM. The cellular enzyme concentration is 10 nM, the rate constants for the enzymatic reaction are $k_1 = 3*10^4 M^{-1}s^{-1}$, $k_{-1} = 1.5\ s^{-1}$, and $k_2 = 18\ s^{-1}$.