Problem Set 3 – Practical Kinetics

BMB 178 Due 12/5/2018 Office Hour: 7-9 pm 12/03 SFL 218

Please turn in the problem set by the end of the class. You can also email to hhsieh@caltech.edu to submit your work.

There are four problems in this section. You may use any books, problem sets, Internet resources, or computer programs to solve these problems. If you get some ideas from a specific paper or website, please cite it as a reference.

Problem 1: Pre-steady state kinetics 1 (30 points)

Polyubiquitination serves as a degradation signal and determines the lifetime of a protein. Ubiquitination is mediated by the sequential reaction of 3 classes of enzymes: a ubiquitin-activating enzyme (E1), a ubiquitin-conjugating enzyme (E2) and a ubiquitin ligase (E3). Read the paper from (doi:10.1038/nature08595) and answer following questions about the mechanism of building polyubiquitin chain on a substrate.

a. Why did the authors need reaction 2 in Figure 1 in addition to reaction 1? What information did reaction 2 give the authors? (8 points)

b. Based on the data in Figure 1, qualitatively describe how the authors arrived at the conclusion that most substrate either undergoes single transfer event or receives single ubiquitin at a time. (10 points)

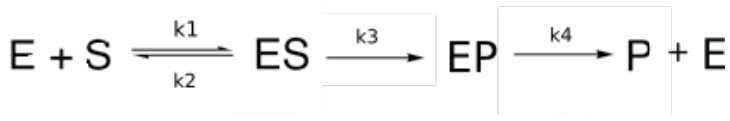
c. What experiments did the authors carry out to distinguish between the sequential vs en bloc models? How did the results led them to conclude that polyubiquitination occurs sequentially? (7 points)

d. Use Berkeley Madonna to simulate the sequential model in Figure 4 and show that it results in the kinetic pattern in Figure 1h. Use the rate constants in Figure 4 for the substrate CYCE, and assume all reactions are pseudo-first order. (5 points)

Problem 2: Pre-steady state kinetics 2 (20 points)

A chemical reaction involving E, S and P is described below:

a. Several sets of reaction conditions and parameters are provided. Choose two sets of reactions in which the kinetic parameters could give rise to a burst phase during the time course of a pre-steady state experiments. For these two sets, indicate which reaction condition gives a single turn-over measurement, and which one is a multiple turn-over measurement. (4 points)



set	$[E]_0$ (nM)	$[S]_0$ (nM)	k_1 ($nM^{-1}s^{-1}$)	k_2 (s^{-1})	k_3 (s^{-1})	k_4 (s^{-1})
1	10	100	0.1	1	1	0.1
2	10	100	0.1	1	0.1	1
3	100	10	0.1	1	0.1	1
4	100	10	0.1	1	1	0.1

b. Use Berkeley Madonna to simulate the two reaction parameter sets you choose from a.

Make sure your units are consistent.

Use startime = 0; stoptime = 50s and t = 0.01s.

Add the script “signal=EP+P” in the equation window, and choose the variable “signal” under the graph option

Save an image of your simulation plot of signal, [EP], and [P] versus time and attach it to your problem set. Make sure to label the graph and select thicker points for easier viewing. (6 points)

c. Compare time traces of signal from single turn-over and multiple turn-over. What accounts for the slow phase in multiple turn-over experiment? Why does the rate of burst phase from multiple turn-over experiment match that of the single turn-over experiment? (6 points)

d. Suppose you are an experimentalist who obtained these data, but do not know the rate constants of the underlying microscopic reaction steps. Describe how you would extract the values of k_3 and k_4 from these data (there are multiple ways to do this, and any solution that works reasonably is fine). (4 points)

Problem 3: Single molecule kinetics (20 points)

φ29 is a model system for investigating viral packaging. This motor packages a 19.3-kilobase pair genome into a capsid that is 40 nm in diameter and 50 nm in height, and can exert forces beyond 60 pN. The motor complex has an ATPase domain that generates the driving force for genome packaging. Each packaging cycle is composed of a dwell phase and a burst phase that results in the translocation of 10 bp of DNA in four 2.5 bp steps. More recently, it was shown that during burst phase, the φ29 motor rotates dsDNA during translocation. Read the paper from Bustamante lab (doi:10.1016/j.cell.2014.02.034) and answer the questions:

a. What are the evidences to show that the motor rotates dsDNA during packaging? (10 points)

b. Under low capsid filling (<50%) conditions, explain the biological significance of dsDNA rotation with respect to the motor. (10 points)

Problem 4: Single molecule kinetics (30 points)

During translation, the ribosome progressively charges aminoacylated tRNA onto mRNA molecule and synthesizes protein. The ribosome contains three tRNA-binding sites corresponding to three adjacent codons. Incoming aminoacylated tRNA is positioned at A site, which is then oriented to react with peptidyl-tRNA in the P site. Deacylated tRNA is released from E site. The movement of ribosome on mRNA is catalyzed by the GTPase EF-G. Read the paper from Puglisi lab (doi:10.1038/nature08925) and answer the following questions:

a. How is fMet-(Cy3)tRNA bound ribosome immobilized in ZMW? (3 points)

b. What would you expect to see if the release of deacylated tRNA from the E site is tightly coupled to the binding of A-site tRNA for $M(FK)_6$? (6 points)

c. Based on the real-time translation of $M(FK)_6$, what seems to be the slowest step during elongation? What could be the reason for that? (8 points)

d. The rate of bacterial translation is 10-20 amino acids per second in vivo. What is the rate of translation measured in 500nM EF-G in this paper? What could contribute to the discrepancy of translation rates measured in this paper from that in vivo? (7 points)

e. List at least two criteria for choosing dye pair for FRET and at least two criteria for dye labeling positions. (6 points)