Problem Set 3 – Practical Kinetics

BMB 178 Due 11/29/2017

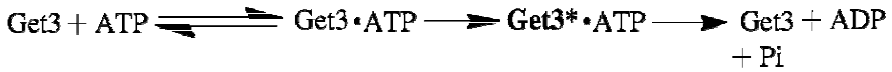
Please turn in the problem set by the end of the class. You can also email to hhsieh@caltech.edu to submit your work.

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 (26 points)

The cytosolic ATPase Get3 mediates targeting of tail-anchored (TA) membrane proteins to the endoplasmic reticulum (ER). Get3 functions as a molecular chaperone by binding to transmembrane domains of newly synthesized TA proteins and shuttling these proteins to a membrane docking complex (Get1/2) in the ER. It has been previously shown through structural studies that the nucleotide state of Get3 regulates its conformation and function. Upon binding ATP, Get3 undergoes a conformational change into a compact "closed" form (denoted as Get3*), which is thought to accommodate the substrate proteins and stimulate ATP hydrolysis. Although Get3 is a dimer, single-site binding and single turnover ATPase assays were done in order to determine the binding constants and nucleotide hydrolysis rates for individual Get3 monomers.

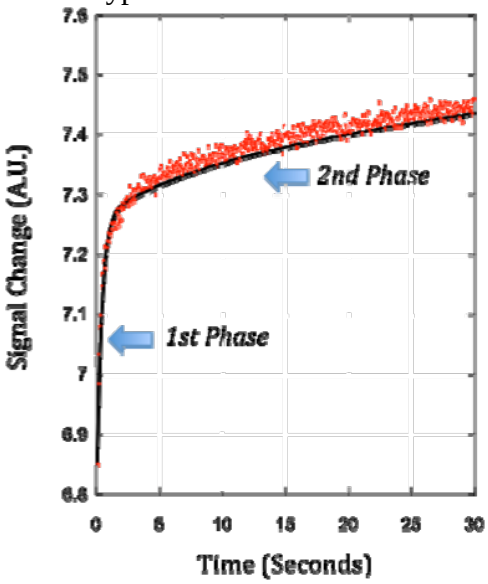
The proposed reaction mechanism for single-site ATP hydrolysis is as follows:



a. The fluorescence of mant-ATP changes upon binding to Get3. Using this assay, the following data was obtained to determine the equilibrium binding constant for the Get3-ATP complex. In this assay, mant-ATP is held constant at 0.4µM and Get3 is titrated. Plot the data below and use non-linear regression to determine the dissociation constant (K_d). (7 points)

[Get3] µM	Fraction Bound
1.6715	0.021889
8.3572	0.14389
16.714	0.31894
25.0	0.38494
33.431	0.50622
41.786	0.55989
90.0	0.75
150.0	0.833
250.0	0.89
400.0	0.93

b. Using the same assay, the following data was obtained with a Stopped-Flow in order to determine the association and dissociation rate constants for nucleotide binding. ATP is held constant at 0.4µM and Get3 is titrated. Shown below is a plot from a typical association rate measurement, which displays two distinct phases.



[Get3] µM	$K_{obsd}(s^{-1})$ 1st Phase	$K_{obsd}(s^{-1})$ 2nd Phase
10	6	0.0032
15	6.6	0.0031
20	7.3	0.0033
25	7.9	0.0032

(1) Propose an explanation for the molecular events that gives rise to both the 1st and 2nd phases. (7 points)

(2) Determine the k_{on} and k_{off} values for the Get3-nucleotide complex by plotting the observed rate constants from the first phase as a function of Get3 concentration. (6 points)

(3) How does this value compare with the dissociation constant from part 1? Does the result support the model you proposed in (1)?

(6 points)

Problem 2: Pre-steady state kinetics 2 (28 points)

a. Assume you mixed 10 nM Enzyme (E) and 1μM substrate (S), and monitored the formation of product (P) as a function of time:

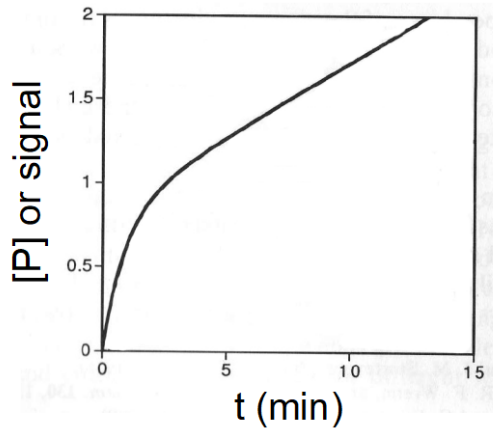


Figure 2A

(1) You can fit the data above to a bi-phasic exponential equation and extrapolate the k_{obsd} for both phases. When you titrate the concentration of substrate and plot the k_{obsd} against substrate concentration, you saw something like this:

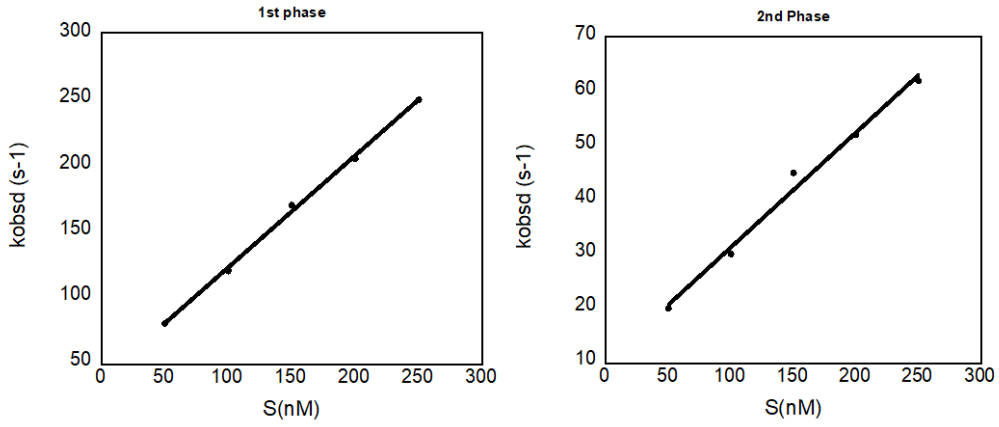
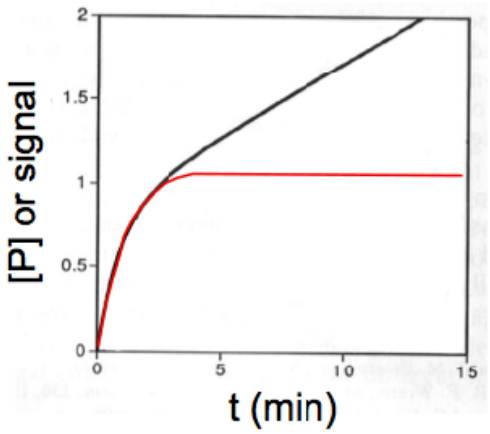


Figure 2B

What could be the model(s) that account for the burst phase? Explain how you draw the conclusion. (6 points)

b. Now you carried out the same experiment for a different reaction involving enzyme E, substrate S, and product P under both single turn-over and multiple turn-over conditions:



Black: multiple turnover
Red: single turnover

Figure 2C

(1) What could be the model that accounts for the burst phase in black line? Explain how you draw the conclusion. (6 points)

(2) A chemical reaction involving E, S and P is described below. Several sets of reaction conditions and parameters are provided. Please choose two sets in which the kinetic parameters of the reaction could give rise to a burst phase during the time course. For these two sets, indicate which reaction condition gives a single turn-over measurement, and which one gives 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

(3) Use Berkeley Madonna to simulate the two reaction parameter sets you choose from (2). (6 points)

Make sure your units are consistent.

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

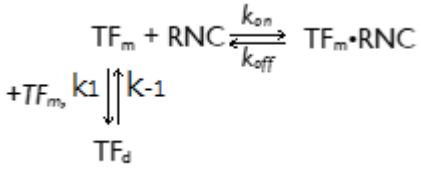
Add the script “signal=EP+P” in the equation window, and choose the variable “signal” under the graph option

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.

(3) Suppose you are an experimentalist who obtained these data, but do not know about 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). (6 points)

Problem 3: Kinetic Simulation (23 points)

TF is a co-translational chaperone. TF’s affinity for translating ribosomes was shown to depend on the length and hydrophobicity of the nascent polypeptide chain on the ribosome. Moreover, TF monomers (TFm) can also dimerize to form homodimers (denoted as TFd). The rate constants during TF dimerization are $k_1 = 0.015\ nM^{-1}\ s^{-1}$ and $k_{-1} = 0.015\ s^{-1}$. A FRET assay has been developed to monitor the interaction of TF with ribosome nascent chain complexes (RNCs). The on and off rates of TF for a specific RNC was determined as shown below:



a. Using a stopped-flow device, the formation kinetics of [TFm-RNC] is monitored. RNC and concentrated TF are quickly mixed and the FRET change of the donor on RNC is recorded. The concentration of TF is high and mixing is rapid, such that TF is >99% in the dimer form immediately after mixing. Thus, the initial conditions of the reaction are: $[RNC]_0$= 20 nM and $[TFd]_0$ = 160 nM. The initial concentrations of other species can be assumed to be 0. The duration of experiment is 20s. The data set is given in the .csv file attached. Use the curve fitting function in Berkeley Madonna to derive k_{on}, k_{off}. (7 points)

Set k_{on} and k_{off} as free variables. If the result does not make sense, you need to play with guess#1 and guess#2 to try different initial conditions. Do not set guess#1 and guess#2 as the same since the software will hit a bug. A good range to play with is 0.001 to 1. Set the tolerance to <0.0001 for accurate result.

In addition to k_{on} and k_{off}, be sure to attach the figure of fitting result to your problem set answer.

b. Using the "batch run" function and the k_{on} and k_{off} you just derived, simulate with a different set of initial conditions: $[RNC]_0$= 20nM, varying $[TFm]_0$ from 10 to 1600 nM. Set the initial concentration of all other species to 0. Monitor the change in [TFmRNC].

(1) Use the geometric series mode and simulate six runs. Keep the runs separate. Increase the stoptime to make sure that you have reached the end point in your simulated reactions. Make sure to attach your graph to your problem set. (5 points)

(2) Plot steady state [TFmRNC] as a function of $[TFm]_0$. Use quadratic equation to determine the apparent K_d. Does this agree with the K_d of TFm for this particular RNC expected from the rate constants? (4 Points)

(3) TFx is a mutation that blocks the dimerization of TF. Perform the same analysis as in (2) and vary $[TFx]_0$ from 10 to 1600 nM and determine the K_d for TF binding to RNC by fitting to quadratic equation. Does this value agree with the K_d of TFm for this particular RNC? (4 Points)

(4) Why are the K_d values obtained in (2) and (3) different? (3 Points).

Problem 4 Single molecule kinetics (23 points)

a. 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:
(1) What are the evidences to show that the motor rotates dsDNA during packaging? (5 points)

(2) Under low capsid filling (<50%) conditions, explain the biological significance of dsDNA rotation with respect to the motor. (5 points)

b. 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:

(1) How is fMet-(Cy3)tRNA bound ribosome immobilized in ZMW? (2 points)

(2) 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? (3 points)

(3) 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? (4 points)

(4) 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? (4 points)