# Global Data Fitting Problem Set

This problem set is based upon fitting a set of 6 experiments contained in the file, "Global_Fit_data.mec". Open the file "Global_Fit_data.mec" using KinTek Explorer. There are six experiments as shown below. Do the analysis and data fitting as described below for each experiment. All concentrations given are those during the reaction after mixing. As you work through each problem, incorporate information gained from previous experiments in each successive experiment, much as you would if the data were collected in the laboratory.
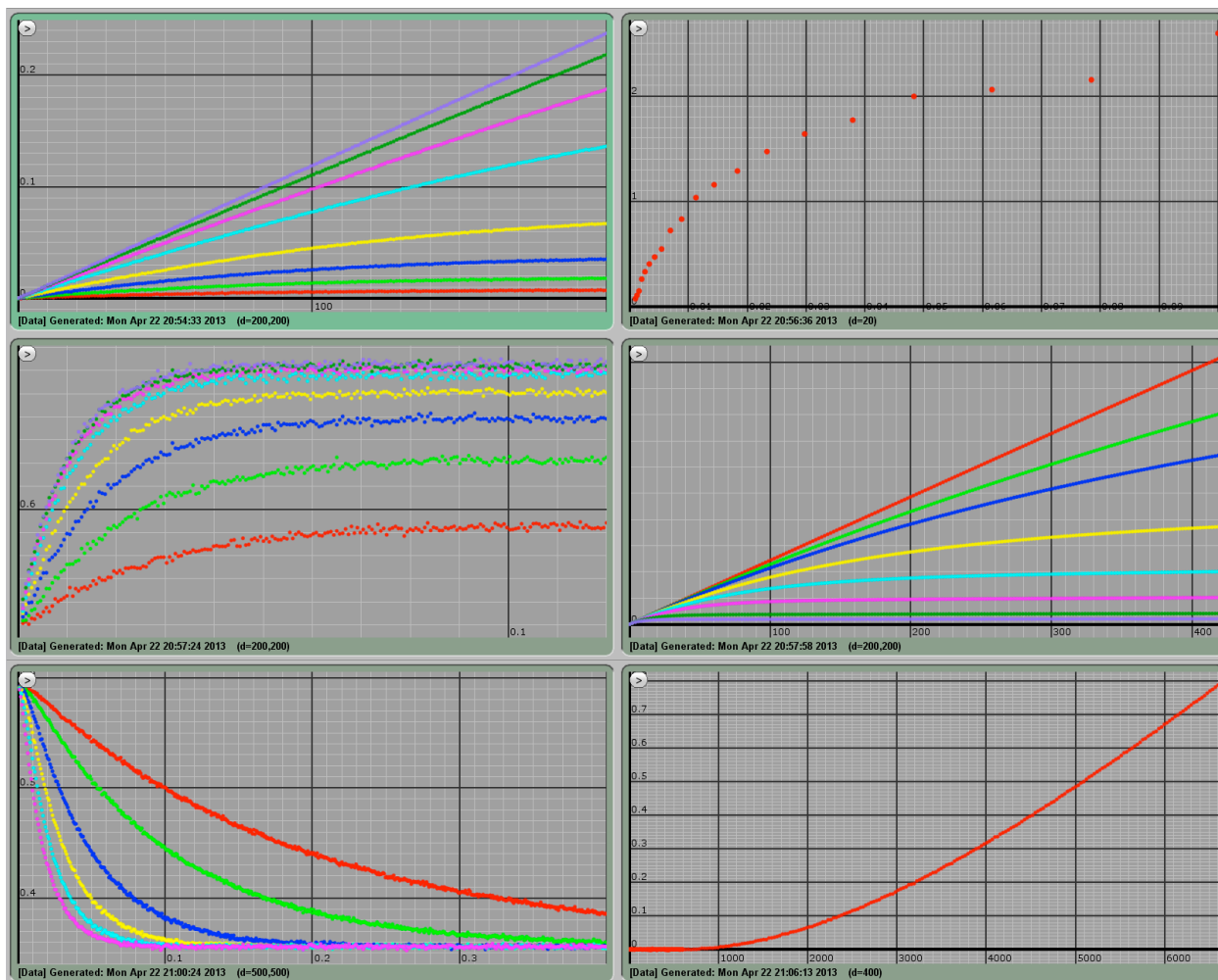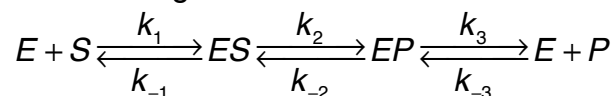
Experiment 1: *Steady state kinetics.* The reaction was initiated by mixing 0.005 µM enzyme with 0.2, 0.5, 1, 2, 5, 10, 20 and 50 µM substrate and then the time course of product formation was monitored by absorbance measurements in a coupled assay system. The data show Absorbance versus time. The products of the reaction yielded extinction coefficient of 0.04 μM^{-1} for the 1 cm pathlength observation cell (ε = 40,000 $M^{-1}cm^{-1}$) used in the experiment.

a. Fit to get the initial rate (aFit) and plot rate versus concentration and fit to the appropriate function to estimate k_{cat} and K_m.
b. Fit the data globally using KinTek Explorer to determine k_{cat} and K_m. You will need to define an appropriate minimal model and output function for the "Observables" under the Experiment Editor, make some simplifying assumptions, and find reasonable starting estimates. What do you learn from fitting these data? What are k_{cat} and K_m values derived from these data? How do they compare to the results from the conventional analysis.
c. Use a print screen (or screen capture) function to save and show your fitted curves from KinTek Explorer.

Experiment 2: *Pre-steady state burst experiment*: The reaction was initiated by mixing 2 µM enzyme with 50 µM radio-labeled substrate, and then quenching with acid at various times shown and determining the concentration of product formed by resolution of substrate versus product by thin layer chromatography.

a. Fit the data using a burst equation using aFit. From the rate and amplitude of the burst and the steady state rate, calculate k_2, k_{-2} and k_3 from the minimal model, assuming that substrate binding is fast:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P$$

b. Enter an appropriate model and fit the data globally using the KinTek Explorer "Fit Active Experiment" function. You will need to define an appropriate output function for the "Observables" under the Experiment Editor and find reasonable starting estimates. Explain your rationale for defining the output and deriving the scaling factors in your fitting. How do the intrinsic rate constants relate to the observed rates derived by fitting to an analytic function (part a)?
c. Use a print screen (or screen capture) function to save and show your fitted curves from KinTek Explorer.

Experiment 3: *Stopped-flow fluorescence experiment*: The reaction was initiated by mixing 1 μM enzyme with various concentrations of substrate (2, 5, 10, 20, 50, 100, 200 and 500 μM), and then using stopped-flow methods to monitor the change in protein fluorescence over time.

    a. Fit the data using an appropriate analytical fitting function (aFit). Explain your choice of the equation for fitting the time dependence of each reaction. What do the constants tell you? What rate constants can you derive?

    b. Graph rate(s) versus concentration of substrate using an external program and fit to an appropriate function. What does the concentration dependence of the rate(s) tell you?

    c. Enter an appropriate model and fit the data globally using the KinTek Explorer "Fit Active Experiment" function. You will need to define an appropriate output function for the "Observables" under the Experiment Editor and find reasonable starting estimates. Explain your rationale for defining the output and deriving the scaling factors in your fitting. How do the intrinsic rate constants relate to the observed rates derived by fitting to an analytic function (part a)?

    d. Use a print screen (or screen capture) function to save and show your fitted curves from KinTek Explorer. What new information have you learned from this experiment?

Experiment 4: *Kinetics of Slow Onset Inhibition*. The reaction was initiated by mixing 0.01 μM enzyme with 100 μM substrate and various concentrations of an inhibitor (0, 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, and 1 μM) and then the time course of product formation was recorded by absorbance measurements as described in Experiment 1.

    a. Fit the data using an appropriate analytical fitting function (aFit). Explain your choice of the equation for fitting the time dependence of each reaction. What do the constants tell you?

    b. Plot (in an external program) rate versus concentration of inhibitor, then fit the concentration dependence of the rate to an appropriate equation in order to derive relevant parameters governing the inhibitor binding reaction. Explain your choice of equation for fitting the concentration dependence of the rate and what the parameters mean.

    c. Enter an appropriate model and fit the data globally using the KinTek Explorer "Fit Active Experiment" function. You will need to define an appropriate output function for the "Observables" under the Experiment Editor and find reasonable starting estimates. Explain your rationale for defining the output and deriving the scaling factors in your fitting. How do the intrinsic rate constants relate to the observed rates derived by fitting to an analytic function (part a)?

    d. Use a print screen (or screen capture) function to save and show your fitted curves from KinTek Explorer. What did you learn in this experiment?

Experiment 5: *Stopped-Flow Kinetics of Inhibitor Binding*. The reaction was initiated by mixing 0.1 μM enzyme with various concentrations of an inhibitor (1, 2, 5, 10, 20 and 50 μM) and then the time course of protein fluorescence was recorded as described in Experiment 3.

a. Fit the data using an appropriate analytical fitting function (aFit). Explain your choice of the equation for fitting the time dependence of each reaction. What do the constants tell you?
b. Plot (in an external program) rate versus concentration of inhibitor, then fit the concentration dependence of the rate to an appropriate equation in order to derive relevant parameters governing the inhibitor binding reaction. Explain your choice of equation for fitting the concentration dependence of the rate and what the parameters mean.
c. Enter an appropriate model and fit the data globally using the KinTek Explorer "Fit Active Experiment" function. You will need to define an appropriate output function for the "Observables" under the Experiment Editor and find reasonable starting estimates. Explain your rationale for defining the output and deriving the scaling factors in your fitting. How do the intrinsic rate constants relate to the observed rates derived by fitting to an analytic function (part a)?
d. Use a print screen (or screen capture) function to save and show your fitted curves from KinTek Explorer. What new information did you learn in this experiment, compared to Experiment 4?

Experiment 6: *Kinetics of Inhibitor Dissociation*. In this experiment the rate of inhibitor dissociation was measured by the regain in enzyme activity as a function of time after dilution of a pre-formed enzyme-inhibitor concentration into substrate. The enzyme-inhibitor complex was formed by incubation of 0.01 μM enzyme with 0.02 μM inhibitor for 600 s. The solution was then diluted 10x into a solution containing 1000 μM substrate and the reaction was monitored by absorbance measurements using the coupled assay as described in Experiment 1 (use the same extinction coefficient).

a. Fit the data using an appropriate analytical fitting function (aFit). Explain your choice of the equation for fitting the time dependence of each reaction. What do the constants tell you?
b. Enter an appropriate model and fit the data using the KinTek Explorer "Fit Active Experiment" function. You will need to set up a multiple mixing experiment in the software (see the instructions and ribozyme.mec experiment 2 for an example) to allow the pre-incubation of enzyme and inhibitor during t1=600 s and then specify the 10x dilution factor in t2 (0.1) with the addition of 1000 μM substrate. When assigning data to the experiment, be sure to uncheck the "Plot all mix steps" box, so that when you assign the data it will be placed only in the t2 reaction time. You also will need to define an appropriate output function for the "Observables" under the Experiment Editor and find reasonable starting estimates. Explain your rationale for defining the output and deriving the scaling factors in your fitting. How do the intrinsic rate constants relate to the observed rates derived by fitting

to an analytic function (part a)? What is the K_d for inhibitor binding? How does this agree with the K_d derived from the slow-onset inhibition data?

c. Use a print screen (or screen capture) function to save and show your fitted curves from KinTek Explorer. What new information did you learn (or better define) in doing this experiment that was not obtained in experiments 4 and 5?

Putting it all together: Fit all five experiments simultaneously to establish the reaction sequence and rate constants.

a. Use screen capture to show the results of your global fitting.
b. Which of the intrinsic rate constants can be derived with confidence from these data? What are the limits of error on the rate constants? You could run the 1D FitSpace to get a better idea of how well constrained each parameter is. Can you derive a single set of rate constants to account for all experiments?
c. At what step(s) in the pathway does the change in protein fluorescence occur?
d. What additional experiment would you want to perform to more accurately establish all the rate constants? Design an experiment and simulate it to show how it would provide additional information or better constraints on rate constants.
e. Is the inhibitor binding a one- or two-step process? What information does the two "inhibitor binding" experiments (Exp. 4 slow onset versus Exp. 5 stopped-flow) bring to bear on the question of one- or two- step binding? If one experiment suggests a one-step binding, while the other shows two-step binding, how can you reconcile the results?