1. Create a model for a simple reaction:

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C$$
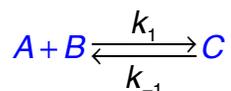
Simulate the formation of C when $[A] = 1$ μM and $[B] = 5$ μM, and with $k_1 = 3$ μM^{-1}s^{-1} and $k_{-1} = 16$ s^{-1}.

a) What is the observed rate of formation of C?
b) How can you explain the observed rate in terms of the intrinsic rate constants?
c) If you were to design an experiment to measure this rate, what time period should you use?
d) Set up a concentration series, measuring the rate of formation of C at various concentrations of B, then plot rate versus concentration. Explain how the concentration dependence defines k_1 and k_{-1}.
e) If you repeat the experiment at different concentrations of A, what do you see?
f) Simulate the reaction where your signal is defined by 0.1*C + 0.6*A. How does that change the reaction amplitude and rate?

2. Modify the reaction in problem 1 to include a second reaction:

$$A + B \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} C \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} D$$
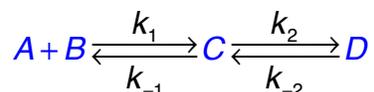
and simulate the loss of A, and the formation of C and D when $[A] = 1$ μM and $[B] = 5$ μM, with $k_1 = 3$ μM^{-1}s^{-1}, $k_{-1} = 16$ s^{-1}, $k_2 = 20$ s^{-1}, $k_{-2} = 2$ s^{-1}.

a) Determine the observed rates of reaction (fit to a double exponential) of A, C, and D. Are the rates the same for each species, and, if so, why?
b) Repeat the calculation at several concentrations of B and plot rate versus concentration for one of the species. Which species do you chose and why? What do you learn from the concentration dependence of the fast and slow rates?
c) Describe how the curves change in shape as you scroll each of the rate constants. For example, I would say, "As k_1 is increased, the rate of decay of A and formation of C increases, while the observed lag time preceding the formation of D decreases, and the amplitude for formation of C increases." Do you agree? What about the other rate constants?

3. Modify the reaction in problem 2 to make the initial binding a rapid equilibrium with the same equilibrium constant, $k_1 = 300$ μM^{-1}s^{-1}, $k_{-1} = 1600$ s^{-1}.
a. How does that change the shape of the curves?
b. Measure the rates of each observable reaction (on the ms – s time scale). What is the rate and how can you explain the observed rate based upon the intrinsic rate constants?

a) Repeat the calculation at several concentrations of B and plot rate versus concentration for one of the species. What does the concentration dependence of the observed rate tell you?
b) What equation would you use to fit the concentration dependence of the observed rate?
c) What are the minimum values of k_1 and k_{-1} (kept in a constant ratio) where you could simply the data fitting for the concentration dependence of the rate of formation of D by assuming a rapid equilibrium binding mode? That is, when is a lag no longer noticeable?

How is this somewhat arbitrary judgment influenced by time resolution, signal/noise ratio and dead time of the instrument?

4. Open the *example* file "binding_data1.mec" and define an appropriate output function, then fit the data. What are the limits of error on the parameters based upon nonlinear regression? Perform a *FitSpace* calculation and compare the error limits with those obtained by simple nonlinear regression.

5. Open the example file "binding_data2.mec". Examine the data and alter the mechanism as needed and define an appropriate output function to fit the data. What are the limits of error on the parameters based upon nonlinear regression? Perform a *Fitspace* calculation and compare the error limits with those obtained by nonlinear regression. How does the shape of the curve depend upon the output factors? Can you get completely different appearance from the same set of rate constants but a different set of output factors? If you link k_1 and k_{-1} to maintain a constant ratio, can you find a lower limit on k_1?

6. Create a model for the binding of a ligand (S) to an enzyme (E) where there is a 16% increase in protein fluorescence upon substrate binding. Enter the output as a*E+b*ES with a=1 and b=1.16. Enter the rate constants shown:

$$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \qquad\qquad k_1 = 5 \ \mu M^{-1}s^{-1}; \ \ k_{-1} = 15 \ s^{-1}$$

a) Simulate an experiment where 2 μM enzyme is mixed with 2, 5, 10, 20, 50, and 100 μM substrate. Click on *Gen Data* under the *Experiment Editor* to generate fake data with sigma = 0.002 and 200 data points per trace over an appropriate time scale for the reaction to go to completion (0.1 s). How does the noise on the data change as you change the sigma value? What is sigma (it is a standard term used in statistics)?

b) Use the aFit function to fit the data to an appropriate analytical function. Click on "Rate vs Conc" to display the concentration dependence of the observed rate(s). Explain the observed concentration dependence in terms of the rate constants. Is there curvature in the plot and, if so, what causes the nonlinearity

c) What happens to the shape of the curves as you change k_1 or k_{-1}? What happens to the "Rate vs Conc" plot?

d) Fit the fake data globally by nonlinear regression to the model. How close are the rate constants to the values originally entered? What are the standard error estimates on the parameters? Is there sufficient information in the data to define all four rate constants and the required output factors?

e) Open the *FitSpace Editor* and *Compute FitSpace*. How do the error limits derived by Fitspace differ from those estimated from the covariance matrix obtained by nonlinear regression (standard error)?

7. Expand the model to include the formation of product alter the output to show a further increase in fluorescence upon forming EP to a level that is 40% greater than E. Edit the output to be a*E+b*ES+c*EP with a=1, b=1.16 and c=1.40. Change the timescale to 0.3 s and generate new fake data as described in Problem 1.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \qquad \begin{aligned} k_1 &= 5 \ \mu M^{-1} s^{-1}; \quad k_{-1} = 15 \ s^{-1} \\ k_2 &= 100 \ s^{-1}; \quad k_{-2} = 25 \ s^{-1} \end{aligned}$$

a) How does the additional step alter the observable kinetics?
b) Use the aFit function to fit the data to an appropriate analytical function. Click on "Rate vs. Conc" to display the concentration dependence of the observed rate(s). Explain the observed concentration dependence in terms of the rate constants.
c) Fit the fake data globally by nonlinear regression to the model. How close are the rate constants to the values originally entered? What are the limits of error on the parameters? Is there sufficient information in the data to define all four rate constants and the required output factors?
d) Open the *FitSpace Editor* and *Compute FitSpace*. How do the error limits derived by Fitspace differ from those estimated from the covariance matrix obtained by nonlinear regression?
e) Click on "Plot observable traces at FitSpace bounds." This will overlay all possible fitted curves computed at the boundaries of the FitSpace parameter boundaries. What does this tell you?

8. Expand the model from the previous problem to include product release.

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E + P \qquad \boxed{\begin{aligned} k_1 &= 5 \ \mu M^{-1} s^{-1}; \quad k_{-1} = 15 \ s^{-1} \\ k_2 &= 100 \ s^{-1}; \quad k_{-2} = 25 \ s^{-1} \\ k_3 &= 5 \ s^{-1}; \quad k_{-3} = 0.1 \ \mu M^{-1} s^{-1} \end{aligned}}$$
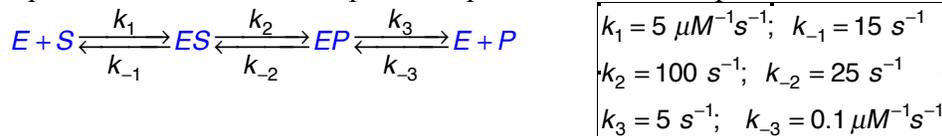
Change time timescale to 0.08 s and generate new fake data as described in Problem 1.
Now click on *New* under the *Experiment Editor* to add a new experiment and enter the initial concentrations of 2 µM enzyme with 100 µM substrate. Define the output to measure the rate of product formation. In this case the output will be the sum of EP and P. Set the timescale to 0.08 s.

a) How do the kinetics differ in comparing experiment 1 (question 2) to experiment 2 (question 3)?
b) Generate fake data to show the burst of product formation with sigma = 0.04 and generating 20 data points.
c) Now fit the data globally including Experiment 1 and Experiment 2. What are the limits of error on the parameters? Is there sufficient information in the data to define all five rate constants and the required output factors?

Compare the standard error based upon nonlinear regression with the limits of error obtained from the FitSpace calculation.

9. Open the file Tryp_synthase_nofit.mec and fit the data.
   a) Use dynamic simulation to fit the data by eye by adjusting each parameter. Be patient and work to understand the relationships between the parameters and the observed traces. Keep working until you have a fit that overlaps the data. Write down the parameters you found. Now press "Fit Active Experiment". How close did you get?
   b) Use the *aFit* function to fit the data to an appropriate analytical function then press "Rate v Conc". What do you learn from the rate versus concentration plot and how could this plot have helped you with starting estimates for paramters.
   c) Return to the results of the global fitting of the data. What are the limits of errors on the parameters? Are these realistic? Perform a *Fitspace* calculation and compare the error limits with those obtained by nonlinear regression.

10. Open the file PNPase-Pf-DADMe-ImmH.mec. The data have already been fit to a model for slow-tight binding inhiibition proposing a two-step inhibitor binding.
   a) Use dynamic simulation and attempt to understand which of the fitted parameters are constrained by the data. Can you discover correlations between parameters; that is, after changing one parameter can you change another parameter to compensate for the changes; or can you link two parameters and find a range over which the two can vary in constant ratio without altering the fitted curves? What do you learn from investigation of the standard error derived from the fit?
   b) Use the *aFit* function to fit the data to the appropriate analytic function. Which function do you chose and why? What do you learn from inspection of the concentration dependence of the rate(s)?
   c) Examine the results of the FitSpace analysis. What do you now learn about the number of parameters that can be derived in fitting the data? Which parameters can be derived with confidence. How can you derive appropriate error limits on the most well-defined parameters? Re-do the FitSpace analysis with fewer parameters after choosing those most constrained by the data. What does this tell you?
   d) Do the data require a two-step inhibitor binding mechanism, or would a simpler, one-step binding be sufficient?

11. Full progress curve kinetics are useful in that an enzyme reaction can be followed to completion to extract k_{cat} and K_m values. It has been claimed that only one progress curve obtained at one concentration of substrate is sufficient and that the data can be fit to a simplified model with two irreversible steps:

$$E + S \xrightarrow{k_1} ES \xrightarrow{k_2} E + P$$
$$k_{cat} = k_2$$
$$K_m = k_2 / k_1$$
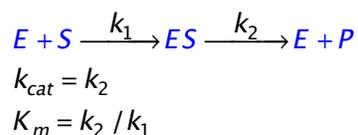
   a) Convince yourself that, theoretically at least, this is a valid shortcut under some limited circumstances.
   b) Determine when this shortcut is invalid. As one approach, you could open the file "Progress_curves.mec" and edit it to obtain the shortcut. One method is to edit the mechanism file to eliminate the explicit ES to EP conversion. Another is to set k_3 to a very large number and set all reverse rate constants to zero. Now attempt to fit the data. Can you fit one progress curve, but not all at the same time? Why is that? What does this tell you? What does the simplified model assume that may not be true?