KinTek Explorer
Sample Exam Questions

Download the latest version of KinTek Explorer from the website (http://www.kintek-corp.com/kinetic_explorer/). In the KinTek Explorer examples folder you will find "Question_1.mec", "Question_2.mec" and "Question_3.mec" containing the data needed to answer the three questions. Data for question 2 in the form of text files for are in the /rawData sub-directory.

Question 1 (15 pts). In the KinTek Explorer examples folder, find "Question_1.mec". The data show the protein fluorescence change following the mixing of an enzyme with an inhibitor. The reaction was initiated by mixing 0.5 μM enzyme with various concentrations of inhibitor (2, 5, 10, 20, 50, 100, 200 μM).

a. Fit the data using the analytical fitting function (aFit) to the appropriate equation. Explain your choice of the equation for fitting the time dependence of the fluorescence change.
b. Plot (in an external program) rate and amplitude 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. What information is available in the amplitude plot?
c. Fit the data globally using the KinTek Exporer "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. Note that the Observable output just has a letter *a* as a placeholder in the downloaded file. Explain your rationale for defining the output and deriving the scaling factors in your fitting.
d. Use a print screen (or screen capture) function to save and show your fitted curves from KinTek Explorer.
e. Which of the intrinsic rate constants can be derived with confidence from these data? What are the limits of error on the rate constants?
f. What additional experiment would you want to perform to more accurately establish all four rate constants?

Question 2 (60 pts). In the KinTek Explorer examples folder, find "Question_2.mec". In addition, data for each of the four experiments can be found in the /rawData directory: Q2A.data.txt, Q2B.data.txt, Q2C.data.txt and Q2D.data.txt. The data are already in the .mec file so will not need to import the data into the program. Note also that the program has default values for rate constants and simple placeholders for outputs. You will need to enter appropriate output factors and estimates for rate constants.

To fit data to equations, you can use either the aFit functions built into KinTek Explorer, or you can import the data into another program for nonlinear regression. In either case, describe the rationale for the equation you chose for fitting and the meaning of the parameters derived as outlined below.

NOTE: If you make an error and loose the data, you will need to re-open the file (this is peculiar to the student version of the software).

As detailed below, fit the data to derive as many kinetic constants as you can for a minimal reaction scheme:

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

Experiment 1 (dataset Q2A.data.txt). A small amount of enzyme (0.01 μM) was mixed with various concentrations of substrate (0.5, 1, 2, 5, and 10 μM) and the time dependence of product formation was monitored until the reaction went to near completion.

a. Fit the data to derive k_{cat} and K_m for the substrate by conventional methods measuring the initial velocity.
b. Fit the data to derive k_{cat} and K_m using the full reaction time course based upon simulation. Using the "Fit Active Experiment" function, find a set of rate constants that fit the data in Experiment 1 without regard to fitting the remaining experiments. Use these constants to compute k_{cat} and K_m. How do these numbers compare to those derived in (a)?
c. Describe how you use these constants to guide you in creating estimates of intrinsic kinetic parameters.

Experiment 2 (dataset Q2B.data.txt). The time dependence of changes in protein fluorescence were recorded after mixing 1 μM enzyme with various concentrations of substrate (2, 5, 10, 20, 50 and 100 μM).

a. Fit the data to an appropriate function (using either aFit function in KinTek Explorer or another program capable of nonlinear regression). Explain why you chose the function you did for fitting data.
b. Plot the rate(s) versus concentration in a separate graph and fit the concentration dependence to extract kinetic parameters. What do the kinetic parameters tell you? Explain the rationale you used in choosing an equation.
c. Describe how these data guide you in entering estimates for intrinsic rate constants in the model.

Experiment 3 (dataset Q2C.data.txt). The time dependence product formation was recorded using rapid chemical-quench flow methods by mixing 1 μM enzyme with 200 μM substrate. The amount of product formed was quantified after quenching the reaction with 1 N HCl at various times.

a. Fit the data to an appropriate function (using either the aFit function in KinTek Explorer or another program capable of nonlinear regression). Explain why you chose the function you did for fitting data. Show your fit to the data.
b. What do the kinetic parameters tell you? Explain the rationale you used in choosing an equation.
c. Describe how these data guide you in entering estimates for intrinsic rate constants in the model.

Experiment 4 (dataset Q2D.data.txt). The time dependence product formation was recorded using rapid chemical quench flow methods after mixing 20 μM enzyme with 2 μM substrate. The amount of product formed was quantified after quenching the reaction with 1 N HCl at various times.

a. Fit the data to an appropriate function (using either aFit function in KinTek Explorer or another program capable of nonlinear regression). Explain why you chose the function you did for fitting data.
b. What do the kinetic parameters tell you? Explain the rationale you used in choosing an equation.
c. Describe how these data guide you in entering estimates for intrinsic rate constants in the model.

Finally, fit all of the data globally to obtain a single set of rate constant to explain all data.

a. Use a print screen (or screen capture) function and show your fitted curves from KinTek Explorer.
b. How many rate constants can be determined?
c. What are the limits of error on each of the rate constants?
d. Compute k_{cat} and K_m, k_{cat}/K_m, K_d for S and K_2 (the equilibrium constant for the chemistry step at the active site) from the final model. Create a table comparing the numbers. How do the values compare to those derived in fitting Experiment 1 by each of the methods?
e. What is the fraction of the enzyme in E, ES and EP during steady state turnover in the presence of saturating substrate concentration?
f. Construct a free energy profile for the pathway. What does this tell you about differences in individual steps in the pathway comparing the two enzymes?

Question 3 (25 pts). Open the file "Question 3.mec" which was obtained for a variant of the enzyme described in Question 2. Note that the experiments included in the file are similar to those described in Question 2, but the time allowed for reaction and some of the concentrations of substrate were different, as described under the Experiment Editor for each experiment.

a. Fit the data globally to derive the rate constants.
b. Use a print screen (or screen capture) function and show your fitted curves from KinTek Explorer.
c. Compute k_{cat} and K_m, k_{cat}/K_m, K_d for S and K_2 (the equilibrium constant for the chemistry step at the active site) from the final model. Create a table comparing enzyme from Question 2 with enzyme from Question 3.
d. Calculate the free energy for substrate binding at a physiological substrate concentration of 5 μM for each enzyme.
e. Is this a better or worse enzyme compared to the enzyme in Question 2? Explain your rationale in describing how the two enzymes differ.
f. Construct a free energy profile for the pathway and use it as a basis for comparing the two enzymes.