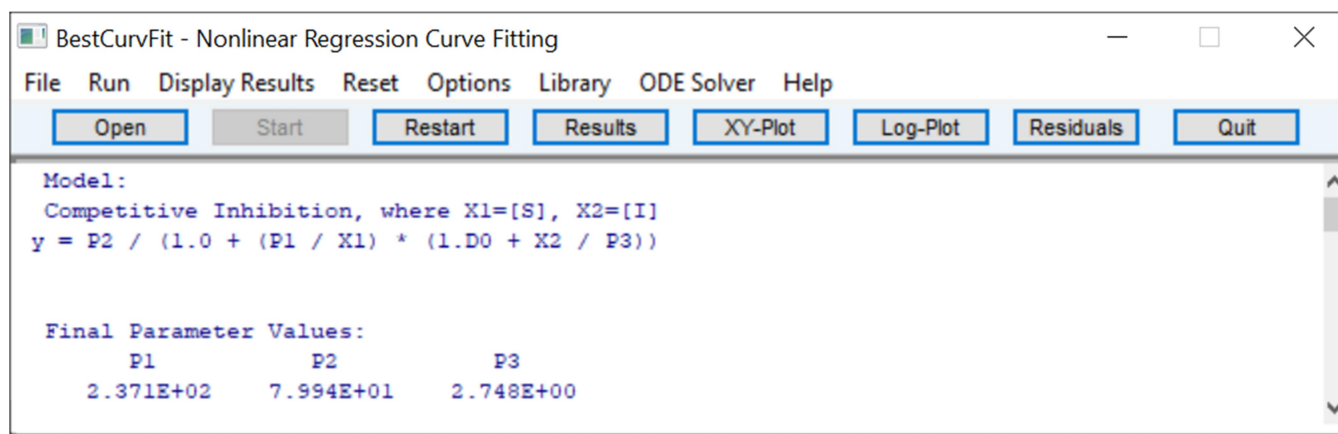


BestCurvFit Enzyme Kinetics
For MS Windows 7.0 or greater

BestCurvFit
Silver Spring, MD 20904
U.S.A

Email: DrFrank88@gmail.com
Internet: <http://www.enzymekinetics.com>



BestCurvFit uses the follow criteria to evaluate the best fit curve to the data.

BestCurvFit uses nonlinear regression to curve-fit data to the chosen model. The program adjusts the model parameters so that the model curve comes as close as possible to the data points. When no further improvement in the fit can be made, the program stops and reports the results of the analysis. Inspection of residual plots is useful to identify systematic deviations of data points from the fitted curve. When deviations or trends are observed, it may suggest that the model is inappropriate. The program uses a Model Information Criterion (MIC) to determine the best curve-fit model. The MIC is a normalized modification of the Akaike information criterion that takes into consideration the sum of squares of residuals, the degrees of freedom of the model, parameter redundancy, outlying data points, and the Runs test of residuals. The smaller the MIC value, including negative values, the better the fitted curve to the data points. The program calculates the approximate standard errors of each fitted parameter of the chosen model. These values should generally not exceed a 25% coefficient of variation [$100 \times (\text{SE}/\text{parameter}) < 25\%$] when the chosen model is a good fit to the data. Parameter values are generally significant when their p-value is less than 0.05 and the eigenvalues are greater than 1.E-05 or 1% of the root-mean-square error. A very large percent principal component can also indicate parameter redundancy. The modified Thompson Tau test is used to find outliers or influential data values. When the Studentized residual exceeds Tau, the data point is considered an influential data point. The Runs test of residuals of the fitted curve to the data is use as a statistical calculation to determine if the data is randomly distributed about the fitted curve at the 95% confidence level. A good fit model to the data should have residuals that are randomly distributed about the fitted curve (the curve Runs through the center of the data points). It is also useful, when fitting functions that are nonlinear in the parameters, to know the degree to which parameters are correlated with each other. Strong correlations can occur when data are fit to a function containing too many parameters, resulting in an over-determined curve-fit. In this case it may be useful to know which parameters should be considered for removal or masking from the model. Since nonlinear least squares models can have objective functions with multiple local minima, curve-fitting algorithms may occasionally converge to local minima depending upon the values of the initial parameter guess, the data noise, and model parameters.

Installing BestCurvFit

Download BestCurvFit Zip file from the Website www.enzymkinetics.com. Select a folder to save the file and then UnZip it. The complete software package will be installed in the directory of your choice. Using MS Windows 7.0 or greater, click on the BestCurvFit.exe file to execute it and display the registration code.

BestCurvFit Software Unlock Code

Once you've installed BestCurvFit on your personal computer, please email (DrFrank88@gmail.com) us your registration code number. Save the code number. You can then either exit the software or turn-off your computer. BestCurvFit will remember the registration code number the next time you run the software. When we receive payment via PayPal on our Website and the registration code number by email, you'll receive in return an activation code number to enter in the space provided on the BestCurvFit Fi unlock menu. Once the activation code is entered, select OK, exit and rerun BestCurvFit. Then you'll have an authorized and fully functional version of BestCurvFit to run on a single computer.

The following files are essential to run BestCurvFit:

BestCurvFit.exe

BestCurvFit.lic

BestCurvFit.ini

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BestCurvFit - Nonlinear Regression Curve-Fitting

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Enzyme Inhibition Analysis

In general, enzyme inhibitors are substances that slow down the rate of reaction of enzymes by preventing either the formation or productive breakdown of enzyme-substrate complex to product.

The derivation of Michaelis-Menten-type inhibition equations is based on several assumptions:

- (1) for enzyme conservation, the total enzyme concentration is equal to the sum of the free enzyme plus the enzyme complexes,
- (2) that the velocity of an enzymatic reaction is proportional to the concentration of the enzyme-substrate complex,
- (3) the substrate concentration must be much greater than the enzyme concentration so that a significant fraction of the substrate is not bound to the enzyme,
- (4) the total substrate concentration approximates the free substrate concentration,
- (5) the initial velocity measurements are taken from the linear portion of the reaction time course curve. The rate observed during this apparent linear period is a measure of the steady-state rate of the reaction,
- (6) enzyme inhibition is a reversible process that does not include enzyme inactivation, which is an irreversible process.

The Michaelis-Menten-type inhibition equations are derived under equilibrium conditions. Under these conditions it is assumed that the enzyme (E), substrate (S), inhibitor (I), and their complexes (ES, EI, EIS) are in equilibrium and that this is maintained during initial velocity studies. It is assumed that all reaction steps are in rapid equilibrium relative to the breakdown of ES and EIS complexes to form product.

The kinetics of inhibition of an enzyme by an inhibitor can be either total or partial. Total inhibitors bind to an enzyme and do not allow the product to form. Inhibitors such as these are referred to as dead-end inhibitors. In comparison, partial inhibitors allow the breakdown of the EIS complex to product. It is assumed that these enzyme-inhibitor complexes form rapidly relative to the other steps in the reaction pathway. The general models of inhibition presented here refer to a particular kinetic behavior of the enzyme and not to any specific mechanism.

Specific compounds that reduce the rate of an enzyme catalyzed reaction are called inhibitors. These chemicals interact reversibly with polypeptide side chains of proteins and inhibit the activity of the enzyme. Reversible inhibitors reach a rapid equilibrium with the enzyme and reduce the rate of the reaction to a constant degree over the time. The equilibrium between the enzyme, substrate, inhibitor, and their complexes (ES, EI, EIS, etc.) is assumed to be instantaneous and maintained during the time it takes to measure the initial velocity. The reaction of an inhibitor with an enzyme should meet the following criteria:

- (1) it should be specific,
- (2) it should inhibit the enzymatic reaction,
- (3) the inhibition should be dose dependent,

- (4) the inhibition should be reversible,
- (5) the inhibitor should not denature the enzyme nonspecifically,
- (6) the equilibrium among inhibitor, substrate, and enzyme complexes is setup instantly.

It is good practice to vary the substrate concentrations over a wide concentration range. At high substrate concentration, relative to the K_m , velocities of reaction are insensitive to changes in the substrate. When the substrate is much less than the K_m , the reaction rate will vary linearly with substrate, and the S/v ratio will approximate the ratio of V_m/K_m . The individual parameters will not be well defined, however. Therefore, varying the substrate over a wide concentration range is good practice. A practical concentration range for the substrate is from $K_m/2$ to $5 \cdot K_m$. Using between 5 and 12 substrate concentrations in this range is usually adequate. This produces a several-fold variation in the velocity measurements from the lowest to the highest substrate concentration.

To determine meaningful kinetic parameters, it is important that accurate initial rates of reaction are measured. The rate of the reaction is the slope of the tangent to the product versus time progress curve. The more the initial portion of the progress curve approaches linearity, the more precise will be the measurement of the initial rate of reaction. Since the rate of an enzyme catalyzed reaction decreases with time of reaction, estimates of the initial rate of reaction may underestimate the true rate. For comparison purposes enzyme inhibitor parameters that are determined for the same enzyme and inhibitor but from several separate independent experiments can be averaged. The averaging of parameter values should be used cautiously, however, so that data is not hidden in mean values. For several K_i values, the mean K_i and standard deviation are calculated as follows:

$$K_i \text{ average} = \frac{\frac{K_{i_1}}{\text{Var}_1} + \frac{K_{i_2}}{\text{Var}_2} + \dots + \frac{K_{i_n}}{\text{Var}_n}}{\frac{1}{\text{Var}_1} + \frac{1}{\text{Var}_2} + \dots + \frac{1}{\text{Var}_n}}$$

$$\text{Std. Dev. average} = \frac{1}{\sqrt{\left(\frac{1}{\text{Var}_1} + \frac{1}{\text{Var}_2} + \dots + \frac{1}{\text{Var}_n}\right)}}$$

where,

K_i = enzyme inhibitor parameter,

$\text{Var}\#$ = variance or square of the standard deviation,

Std. Dev. = standard deviation or squared root of the variance,

In general, the following operational definitions apply to the various types of enzyme inhibition:

- (1) Competitive: inhibitor has no effect on the apparent V_m value,
- (2) Noncompetitive: inhibitor effects V_{\max} but there is no change in apparent K_m value,
- (3) Mixed Noncompetitive: inhibitor effects both apparent V_{\max} and V_m/K_m values,
- (4) Uncompetitive: inhibitor has no effect on the apparent V_m/K_m value.

Enzyme Kinetics

Enzymes play a crucial role in biological processes and are regulated by enhancing or reducing their activity. There are many different kinds of molecules (e.g., drugs) that inhibit enzyme function and various mechanisms exist for enzyme inhibition. Enzyme inhibition is a reduction in the rate of an enzyme-catalyzed reaction by substances called inhibitors. The effects of many drugs are produced as enzyme inhibitors. The determination of enzyme kinetic parameters such as V_{max} , K_m , and K_i are important for the estimation of many biochemical reactions. These include the detection of metabolic control points, intracellular reaction rates, isoenzymes from different tissues, molecular catalysis, and the potency of inhibitors. Enzyme kinetic data is best analyzed by computerized nonlinear least-squares curve-fitting techniques.

Basic considerations in fitting enzyme reactions to models

The initial velocity of an enzyme catalyzed reaction is related to the substrate concentration by the Michaelis-Menten equation,

$$v = \frac{V_{max} * [S]}{K_m + [S]}$$

The K_m and V_{max} parameters define the rectangular hyperbola relating v to S . Three assumptions are important when deriving this equation,

1. the velocity measurement is determined when the concentration of enzyme-substrate complex is not changing,
2. the concentration of enzyme-substrate complex does not significantly deplete the concentration of substrate,
3. the rate of the reverse reaction is negligible.

To measure the initial rate of an enzymatic reaction, the amount of substrate (and coenzyme if needed) must be in excess of the enzyme concentration to ensure that no more than 20% of the available substrate is converted to product. Then the amount of substrate or product formed, will depend only on the amount of enzyme present to catalyze the reaction (i.e., zero-order kinetics). Moreover, there should not be inhibitors present or other substances that may influence the rate of the reaction, and the pH and temperature should be maintained throughout the assay at which the enzyme is active (typically pH 7 to 8 and 25 to 37 °C). When the enzyme is initially introduced to the assay, the excess substrate is steadily combining with free enzyme and the reaction rate increases. When the enzyme becomes saturated with substrate, the rate of product formation, release of free enzyme, and recombining with more substrate proceeds linearly. After this period of time, the reaction rate decreases as the substrate is depleted and the product begins to inhibit the reaction. The reverse reaction may also occur.

Initial velocity measurements are approximated at the linear portion of the curve, representing a zero-order reaction. This is the portion of the $[S]_t$ versus time curve in which the rate is constant with time. As substrate is used up, the enzyme's active sites are no longer saturated, the substrate concentration becomes rate limiting, and then the reaction becomes first order (curved portion). To measure enzyme

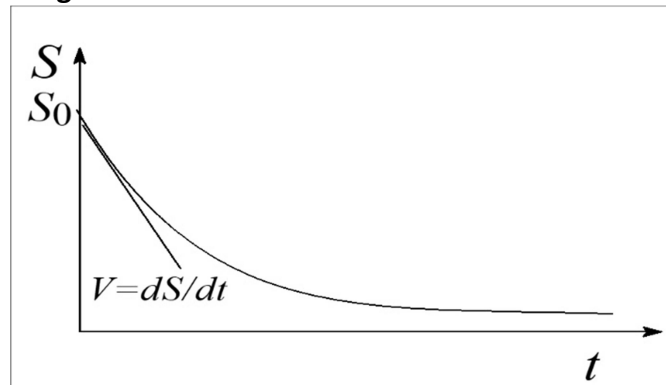
initial rate of reaction (e.g., velocity or activity) accurately, the measurements of substrate depletion or product formation must be made in the portion of the curve where the reaction is zero order. A reaction is most likely to be zero order at the initially start of the reaction since substrate concentration is greatest. To ensure that a reaction is zero order, multiple measurements of substrate (or product) concentration should be made.

When enzyme assays are based on a single measurement at a defined time, it may not be possible to predict the appropriate amount of enzyme to use in fixed-time assays to achieve the optimum velocity. However, this can be empirically determined by dilutions experiment. First, constant volumes of serial 10-fold dilutions of enzyme are assayed to locate the range of dilution in which the calculated activity is maximal (i.e., optimal enzyme concentration for assay). Second, test of the validity of initial velocity assays by determining that the rates observed are a linear function of enzyme concentration. These tests should be applied to all assays in which reaction rates are used to measure enzyme kinetics. BestCurvFit uses Model #18 to globally fit progress curves to estimate initial velocities v_o of each curve.

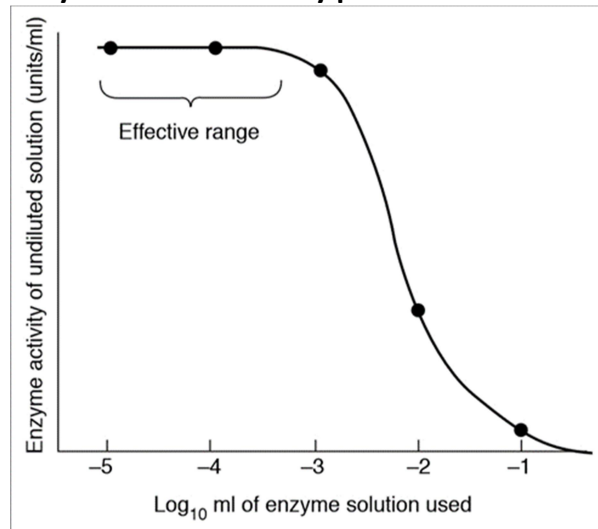
The following principles should be used when estimating enzyme kinetic parameters (Endrenyi,1981):

1. consider some observations at high substrate concentrations when estimating the catalytic constant k_{cat} ;
2. consider observations at low substrate concentrations when estimating competitive inhibition constants K_i , since competitive inhibitors are most effective at low substrate concentrations;
3. consider observations at high substrate concentrations when estimating uncompetitive inhibition constants K_i ;
4. consider very low substrate concentrations when estimating the specificity constant k_{cat}/K_m , although it may not require concentrations to extend to zero;
5. however, consider that the value of the Michaelis constant K_m depends both on k_{cat} and the k_{cat}/K_m , and needs an experimental design that defines both of these.

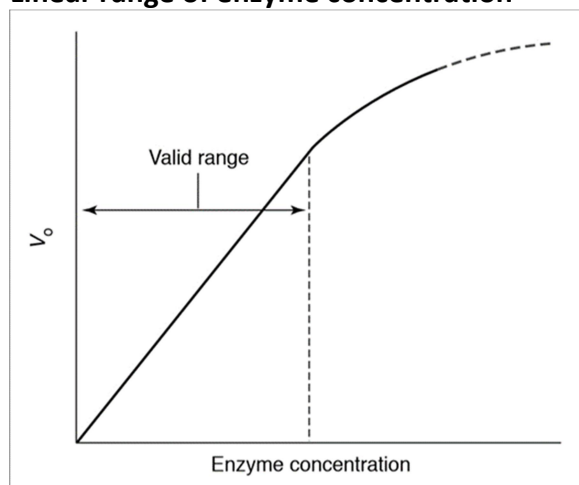
Progress of reaction



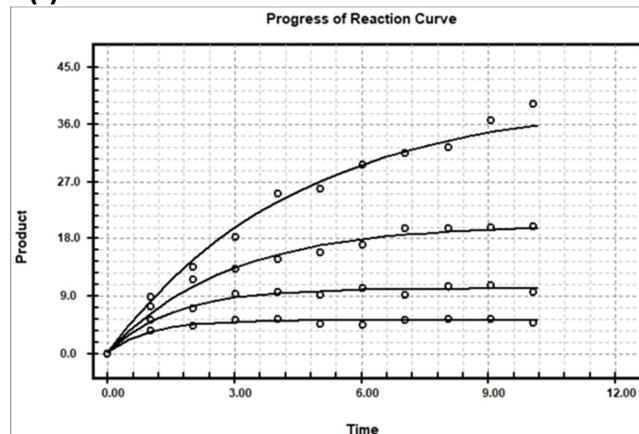
Enzyme dilution activity plot



Linear range of enzyme concentration



Library Model #18 - Enzyme Reaction Progress, P(t) versus Time

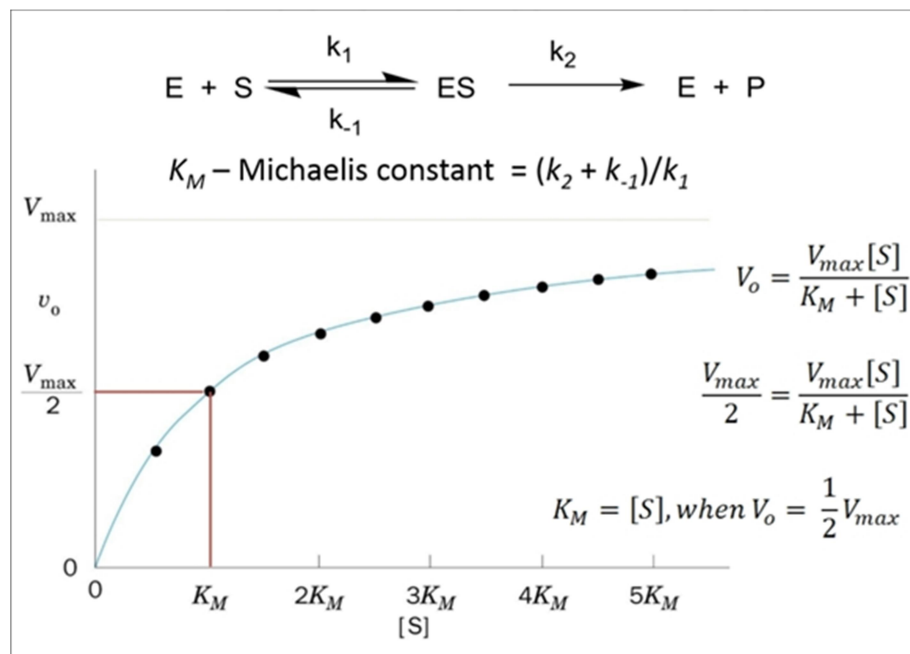


A general strategy for determining K_m , K_i , and V_{max} values

- The basic objective of enzyme kinetics is to maximize the velocity of the enzyme at the minimal enzyme concentration. To achieve this, optimal assay conditions of pH, temperature, ionic strength, and ion concentration need to be established.
- To be certain that a steady-state condition exists, the initial velocity measurements should be made when the velocity is linear both with time and enzyme concentration. If the substrate concentration is depleted substantially or the enzyme concentration is very high, the velocity may not be linear.
- A wide range of substrate and inhibitor concentrations should be used down to the limit of sensitivity of the assay and up to the solubility limit. The substrate range should be at least $K_m/2$ to $5 \cdot K_m$ with at least 5 data points. The inhibitor concentrations should bridge the ranges of $K_m/2$ to $5 \cdot K_m$ with at least 3 different concentration points.
- The standard deviations of the K_m and V_{max} should be below 25% of the mean values. More precise values of K_m and V_{max} may be determined when the substrate concentrations are geometrically spaced.
- When calculating the least-squares fit of the Michaelis-Menten equation to experimental kinetic data, special attention should be given to the following assumptions, (1) the errors in velocity measurements follow a normal distribution, (2) no error exists in the substrate concentration, (3) compensate any systematic bias in the velocity measurements by weighting the points.

In Michaelis-Menten type kinetics, the V_{max} is made up of both the enzyme concentration and a rate constant, and the K_m comprises three rate constants. In practical terms, it is useful to define the V_{max} as the velocity of the reaction when all the substrates are at saturating concentrations, and the K_m as the concentration of substrate required to achieve half the maximum velocity of the enzyme. Nonlinear regression analysis of the Michaelis-Menten hyperbola is the only practical means to obtain reliable kinetic parameters of enzyme catalyzed reactions. The Lineweaver-Burk plot can be used to display

kinetic data, but it should not be used to calculate K_m , K_i , or V_{max} values for enzyme catalyzed reactions. Nonlinear regression curve-fitting is best suited for this purpose.



Enzyme inhibition

Inhibitors can be categorized into four mechanistic types: competitive, uncompetitive, noncompetitive, and mixed noncompetitive.

1. Competitive inhibition occurs when the inhibitor molecules resemble the substrate molecules and bind to the active site of the enzyme. In this way they prevent the normal activity of the enzyme. Competitive type inhibition can be reversed by increasing the concentration of the substrate.
2. Uncompetitive inhibition occurs when the inhibitor binds, not to the free enzyme, but to the enzyme-substrate complex only.
3. In noncompetitive inhibition the inhibitor binds to a part of the enzyme or enzyme-substrate complex other than the active site. This deforms the active site so that the enzyme cannot catalyze the normal reaction. Noncompetitive inhibition cannot be reversed by increasing the concentration of the substrate.
4. Mixed type inhibition is similar to noncompetitive inhibition except that the binding of the substrate or inhibitor to the enzyme affects the binding of the other.
5. Partial inhibition is a variant of the above inhibition types. In this type, the inhibitor only partially prevents the substrate from binding to the enzyme active site and therefore is unable to completely prevent product from being produced. In other words, the inhibitor-substrate-enzyme complex is partially active, and still binds and transforms the substrate although at a lower catalytic rate.

The equations used for the basic types of enzyme inhibition are shown below. However, some practitioners prefer to use IC_{50} values (inhibitory concentration that decreases the activity by 50%) in place of inhibitory constants, K_i 's. The formulas for the conversion of K_i to IC_{50} for the basic types of enzyme inhibition are shown below.

Competitive	$V = \frac{V_{\max}}{1 + \frac{K_m}{[S]} \left(1 + \frac{[I]}{K_i}\right)}$
Non-competitive	$V = \frac{V_{\max}}{\left(1 + \frac{K_m}{[S]}\right) \left(1 + \frac{[I]}{K_i}\right)}$
Un-competitive	$V = \frac{V_{\max}}{\left(1 + \frac{[I]}{K_i^u}\right) + \frac{K_m}{[S]}}$
Mixed	$V = \frac{V_{\max}}{\left(1 + \frac{[I]}{K_i^u}\right) + \frac{K_m}{[S]} \left(1 + \frac{[I]}{K_i^c}\right)}$

Inhibitor Type	IC_{50}	% Inhibition
Competitive	$\frac{K_i}{[I]} \left(1 + \frac{[S]}{K_m}\right)$	$\frac{100}{\frac{K_i}{[I]} \left(1 + \frac{[S]}{K_m}\right)}$
Uncompetitive	$K_i \left(1 + \frac{K_m}{[S]}\right)$	$\frac{100}{\frac{K_i}{[I]} \left(1 + \frac{K_m}{[S]}\right)}$
Noncompetitive	K_i	$\frac{100}{\left(1 + \frac{K_i}{[I]}\right)}$
Mixed	$\frac{[S] + K_m}{\frac{[S]}{K_i^u} + \frac{K_m}{K_i^c}}$	$\frac{100}{\left(\frac{K_m + [S]}{K_i} + \frac{K_m}{K_i^c} + \frac{[S]}{K_i^u}\right)}$

Some criteria used to distinguish among enzyme kinetic models

- A model for which the regression converges but fails to converge for another model, should be accepted over the later model.
- A model for which the parameter values (i.e., K_m , V_{\max} , K_i) produced by the curve-fit give small standard errors, should be accepted over a model that produces very large standard errors of parameter values.
- Models which produce residuals deviations (difference between fitted curve and experimental data) that are relatively small, should be accepted over a model that produces large non-random deviations from the fitted curve.
- Models which produce the smallest Model Information Criterion (MIC) values should be accepted over a model that produce much larger MIC values.
- Models that produce parameter values with standard errors greater than 25%, even if the model may be correct, may have too large a variability of the velocity measurements. Increasing the number of data points may increase the precision of the velocity measurements.
- The distribution of substrate concentrations should be broad enough to span both below and above the K_m value. Similarly, the inhibitors should be used at concentrations that may affect both the K_m and the V_{\max} values.
- When an individual data point is inconsistent with the pattern of the majority of points, such an aberrant point may be considered an outlier. At times, outliers can have a large effect on the parameters and their standard errors. One way of eliminating outliers is to reject them when they are at least two or three standard deviations from the best fitted curve.
- Lastly, velocity measurement errors that arise from experimental protocol or poor technique, pipetting errors, poorly calibrated equipment, enzyme or substrate instability, or by day to day variability should be reduced as much as is practical or if possible eliminated.

Michaelis-Menten Model-Library Model #1

A one substrate reaction scheme was presented by Michaelis and Menten. This model assumes the following:

- (1) the concentration of enzyme is much smaller than the substrate,
- (2) the concentration of product is much smaller than substrate,
- (3) the product-releasing step of the reaction is much slower than the rate at which the substrate dissociates from the enzyme-substrate complex.

$$E + S \rightleftharpoons ES \rightarrow E + P \quad K_s = \frac{[E][S]}{[ES]}$$

$$y = \frac{(V_{max} * [S])}{(K_m + [S])}$$

Hill Sigmoid Model-Library Model #2

The Hill model was first used for the analysis of the binding of oxygen to hemoglobin. The model was used to represent the binding of substrate with several equivalent sites on an enzyme. Typically, the model is used to represent sigmoid (S-curve) or cooperative dose-response relationships. The following reaction scheme describes the model:

$$E + nS \rightleftharpoons ES_n \rightarrow E + P \quad K_{50}^n = \frac{[ES_n]}{[E][S]^n}$$

$$y = \frac{V_{max}}{1.0 + \left(\frac{K_{50}}{[S]}\right)^n}$$

where n represents the apparent number of substrate binding sites per enzyme, but also may represent the degree of interaction between them. For example, if n were to equal a value around two, this could indicate at least two binding sites with good cooperativity or it could just as well represent four sites with poor cooperativity. The K50 represents the substrate concentration that yields the half-maximal velocity.

Four Parameter Logistic Model-Library Model #22

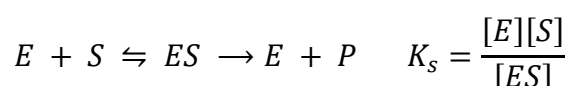
Dose-response relationships for enzyme inhibition or activation, as well as bioassays typically are represented by a smooth declining or increasing curve. These curves usually can be represented by a logistic model. A general form of the logistic function is the Four-Parameter Logistic model:

$$Y = \frac{a - d}{1 + \left(\frac{X}{c}\right)^b} + d$$

where Y is the response and X is the dose variables. The parameter "a" is the response when X = 0, "d" the response at infinite dose, and "c" is the ED₅₀ or IC₅₀ of the dose producing a response halfway between "a" and "d". The slope factor "b" represents the steepness of the dose-response curve and should not be interpreted as a Hill coefficient.

Competitive Inhibition Model-Library Model #4

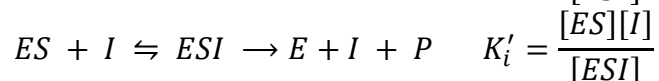
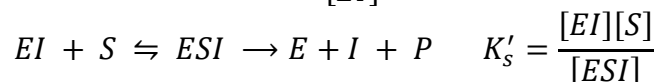
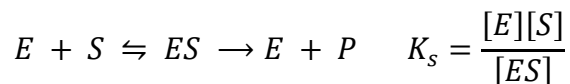
Typically, substrate analogues are competitive inhibitors. Competitive inhibitors may be structural analogues whereby the inhibitor binds to the same amino groups on the protein surface as the substrate. The inhibitor occupies the same binding site as the substrate so that the substrate cannot bind and the enzyme activity is reduced. When the substrate and an analogue compete for the same substrate binding site on the protein, it is called competitive inhibition. During competitive inhibition, the substrate analogue or inhibitor does not affect the rate of breakdown of the enzyme-substrate complex. When the substrate is much in excess, the maximal velocity is still obtained in the presence or absence of inhibitor. A competitive inhibitor does affect the Michaelis or dissociation constant. The inhibitor interacts with groups of the protein that participate in the binding of the substrate and alters the tightness of substrate binding and reduces the binding of the enzyme to substrate molecules.



$$y = \frac{V_{max}}{1.0 + \left(\frac{K_s}{[S]}\right) * \left(1.0 + \frac{[I]}{K_i}\right)}$$

Partial Competitive Inhibition Model-Library Model #8

The inhibitor acts as a Competitive inhibitor (see Competitive Topic) but does not completely block the substrate binding site. As a result, the inhibitor is not capable of completely blocking, even at high concentrations, the binding of substrate to the enzyme active site. Both Competitive and Partially Competitive inhibitors produce the same Lineweaver-Burk and Eadie-Hofstee graphs when plotted by linear regression. However, nonlinear regression analyses show that the distance between lines of a Lineweaver-Burk plot changes with inhibitor concentration representing different strengths of reaction between the inhibitor and the substrate for the binding site.

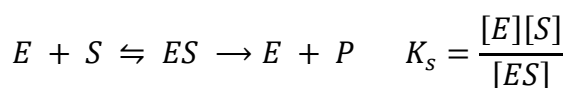


$$\frac{K_i}{K'_i} = \frac{K_s}{K'_s}$$

$$y = \frac{V_{max}}{1.0 + \left(\frac{K_S}{[S]}\right) * \left(\frac{1.0 + \frac{[I]}{K_i}}{1.0 + \frac{[I]}{\alpha * K_i}}\right)}$$

Noncompetitive Inhibition Model-Library Model #5

When an inhibitor does not affect the dissociation constant of the enzyme-substrate complex but affects the maximal velocity of the reaction, then it is a Noncompetitive inhibitor. Since the dissociation constant is unchanged in the presence of inhibitor, the inhibitor is believed to bind to a different site than the substrate. That is the substrate and inhibitor do not influence each other's binding to the enzyme. This implies that the catalytic site and the inhibitor binding sites are different. However, the inhibitor binding site and the catalytic sites could overlap.

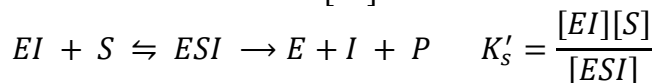
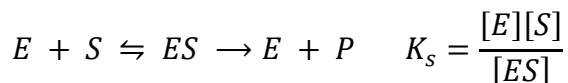


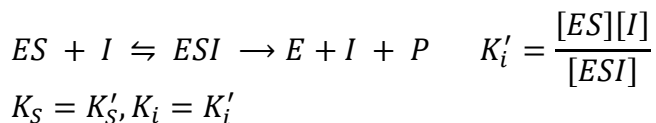
$$K_s = K'_s, K_i = K'_i$$

$$y = \frac{V_{max}}{\left(1.0 + \frac{K_S}{[S]}\right) * \left(1.0 + \frac{[I]}{K_i}\right)}$$

Partial Noncompetitive Inhibition Model-Library Model #9

If the substrate can still catalyze a reaction but at a lower rate when the inhibitor is present is it called Partially Noncompetitive inhibition (see also Noncompetitive Topic). Both Noncompetitive and Partially Noncompetitive inhibitors produce the same Lineweaver-Burk and Eadie-Hofstee graphs when plotted by linear regression. However, nonlinear regression analyses show that the distance between lines of a Lineweaver-Burk plot changes with inhibitor concentration representing different strengths of reaction between the inhibitor binding and the substrate catalyzed reaction.

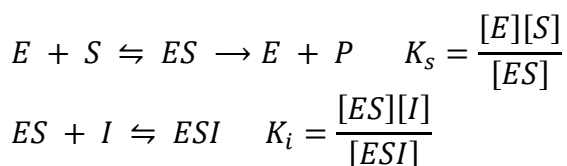




$$y = \frac{V_{max}}{\left(\frac{1.0 + \frac{[I]}{K_i}}{1.0 + \beta * \frac{[I]}{K_i}} \right) + \left(\frac{K_S}{[S]} \right) * \left(\frac{1.0 + \frac{[I]}{K_i}}{1.0 + \beta * \frac{[I]}{K_i}} \right)}$$

Uncompetitive Inhibition Model-Library Model #6

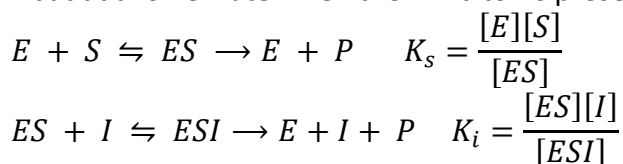
When the inhibitor is not able to bind to the enzyme except when the substrate is bound in an enzyme-substrate complex, then it is called Uncompetitive inhibition. The inhibitor combines with the enzyme substrate complex and facilitates the binding of the substrate thereby lowering the K_m . In uncompetitive inhibition, the inhibitor decreases both the dissociation constant of the substrate and the maximal velocity of the reaction.



$$y = \frac{V_{max}}{\left(\frac{K_S}{[S]} \right) + \left(1.0 + \frac{[I]}{K_i} \right)}$$

Partial Uncompetitive Inhibition Model-Library Model #10

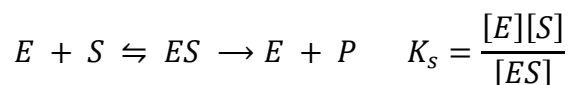
When the inhibitor is not able to bind to the enzyme except when the substrate is bound in an enzyme-substrate complex, then it is called Uncompetitive inhibition. The substrate can still catalyze a reaction but at a lower rate when the inhibitor is present.



$$y = \frac{V_{max}}{\left(\frac{1.0 + \frac{[I]}{\alpha * K_i}}{1.0 + \frac{[I]}{K_i}} \right) + \left(\frac{K_S}{[S]} \right)}$$

Mixed Noncompetitive Inhibition Model-Library Model #7

Mixed type inhibition consists of both competitive and noncompetitive inhibition mechanisms when $K'_i > K_i$. Another form of mixed inhibition occurs when the $K'_i < K_i$ and noncompetitive and uncompetitive type inhibition patterns occur. Mixed type inhibitors increase the dissociation constant of the substrate and reduce the maximal reaction velocity.

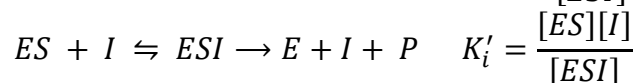
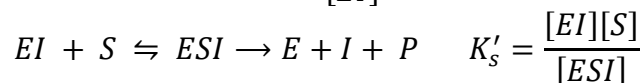
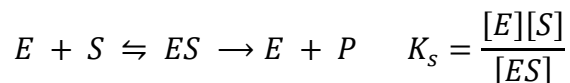


$$K'_s = K_s * \frac{K'_i}{K_i}$$

$$y = \frac{V_{max}}{\left(\frac{K_s}{[S]}\right) * \left(1.0 + \frac{[I]}{K_i}\right) + \left(1.0 + \frac{[I]}{K'_i}\right)}$$

Partial Mixed Noncompetitive Inhibition Model-Library Model #11

Another form of mixed inhibition occurs when the enzyme-substrate inhibitor complex can break down to product but with a rate less than that of the enzyme-substrate complex alone. Mixed type inhibitors alter the dissociation constant of the substrate and reduce the maximal reaction velocity.



$$K'_s = K_s * \frac{K'_i}{K_i}$$

$$y = \frac{V_{max}}{\left(\frac{1.0 + \frac{[I]}{a * K_i}}{1.0 + b * \frac{[I]}{a * K_i}}\right) + \left(\frac{K_s}{[S]}\right) * \left(\frac{1.0 + \frac{[I]}{K_i}}{1.0 + b * \frac{[I]}{a * K_i}}\right)}$$

What is Mixed Type Inhibition?

Enzyme inhibition of a reversible nature can be divided into two main types, complete and partial inhibition. This is also known as linear and hyperbolic inhibition, respectively. BestCurvFit only addresses the inhibitory effects that arise when compounds react reversibly with the enzyme. It is assumed that Michaelis Menten assumptions apply to both the substrate and inhibitor. With complete inhibition, the velocity approaches zero as the concentration of inhibitor increases. With partial inhibition the enzyme is still capable of converting substrate to product but at a reduced rate or partially because the ESI complex can break down to form product $ESI \rightarrow E + I + P$.

Mixed inhibition is a reversible form of inhibition where the substrate and inhibitor can affect the binding of other species such as ES and EIS complexes.

$$v = \frac{V_{max} * [S]}{K_S * \left(1 + \frac{[I]}{K_i}\right) + [S] * \left(1 + \frac{[I]}{K'_i}\right)}$$

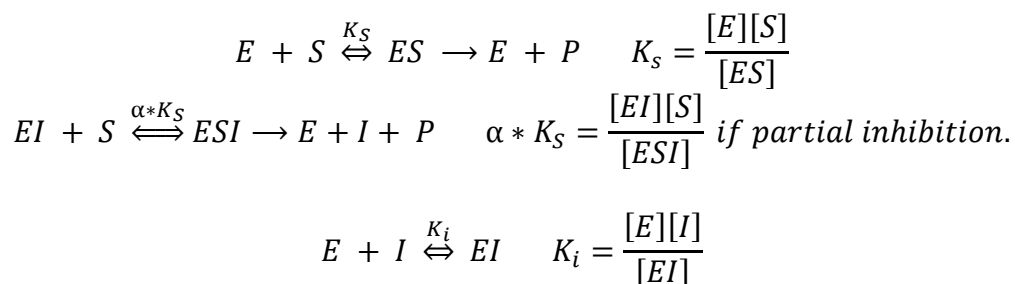
or

$$v = \frac{V_{max} * [S]}{K_S * \left(1 + \frac{[I]}{K_i}\right) + [S] * \left(1 + \frac{[I]}{\alpha * K_i}\right)}$$

For mixed type inhibition the degree of inhibition depends on the magnitudes of K_i and K_{i2} . If $K_{i2} = \alpha * K_i > K_i$ (where $\alpha > 1$), then the inhibition contains both competitive and noncompetitive type inhibition. Therefore, the extent of inhibition is reduced or overcome by increasing the substrate concentration. The inhibition will not be completely reversed by excess substrate (saturated) unless the $K_{i2} = \alpha * K_i \gg K_i$ (where $\alpha \gg 1$), in which case the mechanism of inhibition reduces to that of competitive inhibition.

In contrast, if $K_i > K_{i2} = \alpha * K_i$ (where $\alpha < 1$), then the inhibition will contain noncompetitive and uncompetitive mechanisms. The amount of inhibition will then increase by an increase in the substrate concentration. The mechanism of inhibition will reduce to pure uncompetitive when $K_i \gg K_{i2} = \alpha * K_i$ (where $\alpha \ll 1$).

The mechanism of Mixed type inhibition can be extended further to allow for the breakdown of the EIS complex to product, as in partial mixed inhibition.



$$ES + I \xrightleftharpoons{\alpha * K_i} ESI \quad \alpha * K_i = \frac{[ES][I]}{[ESI]}$$

$$(\alpha * K_S) * K_i \approx (\alpha * K_i) * K_S$$

$$\alpha * K_i = \frac{[E][I]}{[EI]} = \frac{[ES][I]}{[ESI]}$$

If $\alpha = 1$ the mechanism reverts to noncompetitive inhibition. In other words, noncompetitive inhibition is a special case of mixed type when the value of α equals one. It is possible in Mixed inhibition models to use two separate inhibition constants, K_i and K_{i2} or K_i and $K_{i'}$. For example, K_{i2} or $K_{i'}$ can be calculated simply as $K_{i2} = K_{i'} = \alpha * K_i$. The behavior of the mechanism changes as the parameter α varies. When α is in the range of infinity $> \alpha > 1$ the mechanism is mixed type inhibition.

How to Use BestCurvFit

Steps to run the analysis:

1. Create an external text data file containing:
 - 1.1 1st line: Model # or User-define function
 - 1.2 2nd line: Parameter values
 - 1.3 3rd line: Lower bounds of parameter values
 - 1.4 4th line: Upper bounds of parameter values
 - 1.5 5th line: TRUE or FALSE mask of parameter (Fixed=FALSE)
 - 1.5.1 Masks can be FALSE, F, false, or f
 - 1.6 6th line: X,Y data in columns
 - 1.7 7th line: Word END is entered after the last data row
2. The delimiters for data file values are a space or comma.
3. UDF parameters (P1..P8) must be in sequence (Do not skip one)
4. Independent variables (X1..X3) must also be in sequence
 - 4.1 UDF(X1,X2, P1,P2) or UDF(X1, P1,P2,P3) is allowed
 - 4.2 UDF(X1,X3, P1,P2) or UDF(X1, P1,P2,P4) is NOT allowed
5. Enter data by selecting the 'File' option to load data from a file. Always enter at least one data curve. For example, the first curve may contain several data pairs (e.g., velocity, substrate concentration) and optionally an inhibitor concentration value.
6. Options: For hard to fit models choose DIFFICULT (default=EASY)
7. Options: For weighting of data: Reweight, $1/y^2$, or Robust weight
8. Select RUN to execute curve-fitting of the selected data file.
9. Scroll down to review the results of the curve-fit.
10. Select Display Results to review the goodness-of-fit statistics, parameter values, standard errors, and correlation matrix.
11. Select Plot to display the plotted data or residuals.

BestCurvFit Functions and Operators**MATHEMATICAL:**

(Addition, Subtraction, Multiplication, Division, Power)

+, -, *, /, ^ or **, and parentheses

CONDITIONAL EXPRESSIONS:

(If True value is 1, otherwise 0 Value If False)

equal, = (P1=P2)

not equal, # (P1#P2)

less than, < (P1<P2)

greater than, > (P1>P2)

less than or equal to, [(P1[P2)

greater than or equal to,] (P1]P2)

MAXIMUM/MINIMUM VALUE:

(the lesser of P1 or P2)

minimum(a,b), : (P1:P2)

(the greater of P1 or P2)

maximum(a,b), ! (P1!P2)

FUNCTIONS:

All functions take a single numeric argument enclosed in parentheses.

Algebraic: ABS(), SQRT()

Transcendental: EXP(), LOG10(), LOG() [natural]

Trigonometric: SIN(), COS(), TAN()

Inverse Trig: ASIN(), ACOS(), ATAN()

Hyperbolic: SINH(), COSH(), TANH()

Lambert Omega W: OMEGW()

CONSTANTS:

Pi = Pi constant: 3.1415926535897932

VL = lower limit: 1E-16

VH = upper limit: 1E+16

RC = gas constant: 1.9872041 cal/(K*mol)

RJ = gas constant: 8.3144621 J/(K*mol)

K1 = degree Kelvin: 273.15 °K (0 °C Celsius)

K2 = degree Kelvin: 298.15 °K (25 °C Celsius)

DG = Convert radians to degrees: 57.2957795130823208

TEMPERATURE:

Temperature (°F) = 1.8 * Temperature (°C) + 32

Temperature (°K) = Temperature (°C) + 273.15

Temperature (°C) = (Temperature (°F) - 32) / 1.8

Temperature (°C) = Temperature (°K) - 273.15

Note: This program is not case-sensitive, so you can refer to the variables as x1 or X1, and the parameters as p1, P1, p2, or P2, etc.; and the function names can be written as (for square root, for example) sqrt, SQRT, or Sqrt. Constants: Pi [=3.14...], Dg [=180/Pi = 57.2957795130823208]

Note: The trig functions work in radians. For degrees, multiply or divide by the Deg variable. For example: SIN(30/Dg) will return 0.5, and ATAN(1)*Dg will return 45.

BestCurvFit Internal Library of Models:

A library of models is included in BestCurvFit to facilitate the analysis of enzyme kinetic models, or you may create custom User-defined (UDF) equations for curve-fitting data to a model. A UDF can have up to 8 parameters (P1...P8) and 3 independent X1, X2, and X3 variables. BestCurvFit provides some references for for models in the menu 'Library/Library Models-#/Help'.

Basic enzyme kinetics reference books:

1. Enzyme Kinetics - A Modern Approach, A. G. Marangoni
Search Web for available pdf:
<http://www.enzymes.at/download/Enzyme%20Kinetics%20-%20A%20Modern%20Approach.pdf>
2. Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis, R. A. Copeland (2000); Search Web for available pdf:
<https://enzimo.files.wordpress.com/2012/09/enzymes-a-practical-guide2.pdf>
3. Enzyme Kinetics, I. H. Segel, John Wiley & Sons, NY (1975)
4. Kinetic Data Analysis, L. Endrenyi, Plenum Press, NY (1981)

BestCurvFit Library of Enzyme Kinetic Models:

- Model 1 - Michaelis-Menten
- Model 2 - One Site Sigmoidal Hill
- Model 3 - Two Site Michaelis-Menten
- Model 4 - Competitive Inhibition
- Model 5 - Noncompetitive Inhibition
- Model 6 - Uncompetitive Inhibition
- Model 7 - Mixed Noncompetitive Inhibition
- Model 8 - Partial Competitive Inhibition
- Model 9 - Partial Noncompetitive Inhibition
- Model 10 - Partial Uncompetitive Inhibition
- Model 11 - Partial Mixed Noncompetitive Inhibition
- Model 12 - Random Bi-Bi 2 Substrate
- Model 13 - Ordered Bi-Bi 2 Substrate

Model 14 - Ping Pong Bi-Bi 2 Substrate
 Model 15 - Two Substrates: One Enzyme, One Active Site
 Model 16 - Two Substrates: One Enzyme, Two Active Sites
 Model 17 - Enzyme Initial Exponential Progress Curve
 Model 18 - Enzyme Initial Exponential Multi-Progress Curves
 Model 19 - Quasi-S-S Integrated Michaelis-Menten minus baseline - 1
 Model 20 - Quasi-S-S Integrated M-M [So] minus baseline - 2
 Model 21 - Yonetani-Theorell Mutual Exclusivity of Inhibitors
 Model 22 - Four-Parameter Logistic
 Model 23 - Five-Parameter Logistic
 Model 24 - Descending Exponential
 Model 25 - Ascending Exponential
 Model 26 - Positive or Negative Substrate Cooperativity
 Model 27 - Arrhenius 1st-Order Substance Stability (k)
 Model 28 - Arrhenius 1st-Order Substance Stability (t90)
 Model 29 - Arrhenius Zero-Order Substance Stability (k)
 Model 30 - Arrhenius Zero-Order Substance Stability (t90)
 Model 31 - Arrhenius 2nd-Order Substance Stability (k)
 Model 32 - Arrhenius 2nd-Order Substance Stability (t90)
 Model 33 - Integrated M-M Competitive Inhibition
 Model 34 - Integrated M-M Uncompetitive Inhibition
 Model 35 - Integrated M-M Noncompetitive Inhibition
 Model 36 - Integrated Michaelis-Menten Mixed Inhibition.
 Model 37 - Botts-Morales General Modifier Model
 Model 38 - Baici Tight-binding Hyperbolic Noncompetitive Model
 Model 39 - Baici Tight-binding Hyperbolic Competitive Model
 Model 40 - Baici Tight-binding Modifier 6-parameterModel
 Model 41 - Gaddum-Schild Logistic Agonist/Antagonist Model
 Model 42 - Botts-Morales Hyperbolic Substrate Inhibition Model (Vmax)
 Model 43 - Botts-Morales Hyperbolic Substrate Inhibition Model (Ymax)
 Model 44 - Hyperbolic Substrate/Competitive Inhibition Model (Ymax)
 Model 45 - Hyperbolic Substrate/Uncompetitive Inhibition Model (Ymax)
 Model 46 - Hyperbolic Substrate/Noncompetitive Inhibition Model (Ymax)

Library Models for Curve-fitting:

There are 46 library models to choose from for curve-fitting data.

- I. The first three models (#1, 2, 3) are non-inhibition models. They include the Michaelis-Menten, Hill, and Two Site Michaelis-Menten models for hyperbolic, sigmoidal, and biphasic dose-response or substrate versus velocity curves, respectively.

$$y = \frac{(V_{max}*[S])}{(K_m+[S])}, \quad y = \frac{(V_{max}*[S]^n)}{(K_m^n+[S]^n)}, \quad y = \frac{(V_{max1}*[S])}{(K_{m1}+[S])} + \frac{(V_{max2}*[S])}{(K_{m2}+[S])}$$

- II. The next 8 models (#4, 5, 6, 7, 8, 9, 10, 11) are enzyme inhibition models which include: Competitive inhibition, Noncompetitive inhibition, Uncompetitive inhibition, Mixed Noncompetitive inhibition, Partially Competitive inhibition, Partially Noncompetitive inhibition, Partially Uncompetitive inhibition, and Partially Mixed Noncompetitive inhibition.

e.g., Noncompetitive Inhibition:
$$y = \frac{V_{max}}{\left(1.0 + \frac{K_m}{[S]}\right) \left(1.0 + \frac{[I]}{K_i}\right)}$$

- III. The next 3 models (#12, 13, 14) are two substrate bi-bi models:
Random Bi-Bi 2 Substrate, Ordered Bi-Bi 2 Substrate, and Ping Pong Bi-Bi 2 Substrate

e.g., Rapid Equilibrium Random bi-bi:
$$y = \frac{V_{max}}{1.0 + \left(\frac{K_b}{[B]}\right) + \left(\frac{K_a}{[A]}\right) + \left(\frac{K_{ia} \cdot K_b}{[A] \cdot [B]}\right)}$$

- IV. The next 2 models (#15, 16) are two substrate models for one or two active sites:
One Enzyme, One Active Site, One Enzyme, Two Active Sites

Two Substrates: One Enzyme, One Active Sites, where X1=[A], X2=[B]

$$y = \frac{V_{max1} * K_{m2} * [A] + V_{max2} * K_{m1} * [B]}{K_{m1} * K_{m2} + K_{m1} * [B] + K_{m2} * [A]}$$

Two Substrates: One Enzyme, Two Active Sites, where X1=[A], X2=[B]

$$y = \frac{V_{max1} * K_{m2} * [A] + V_{max2} * K_{m1} * [B] + (V_{max1} + V_{max2}) * [A] * [B]}{K_{m1} * K_{m2} + K_{m1} * [B] + K_{m2} * [A] + [A] * [B]}$$

- V. The next 2 models (#17 and 18) are product of reaction models (progress curves):
Enzyme Initial Exponential Progress Curve minus background, and Enzyme Initial Exponential Multi- 7 Progress Curves. These models calculate the initial velocity (vo) of progress curves. The rate constant k adjusts for nonlinearity.

Model #17:
$$y = P_t = v_o/k * (1 - EXP(-k * t)) + Bkg$$

NOTE: The data file requires time (t) and product y (Pt) data columns for model #17, and time, index number of curve, and product y (Pt) data columns for model #18.

- VI. The next 2 models (#19 and 20) are Quasi-Steady-State Integrated Michaelis-Menten models:
The first subtracts a baseline parameter, and the second estimates the [So] as a parameter in the model and subtracts a baseline for single substrate progress of reaction models:

e.g., Integrated Michaelis-Menten minus baseline (Model #19)

$$y = P_t + Bkg = ([S]_o - [S]_t) + Bkg ,$$

$$\text{where } [S]_t = K_m * \text{Omega}W(W)$$

$$y = ([S]_o - K_m * \text{Omega}W(W)) + Bkg$$

$$W = \left(\frac{[S]_o}{K_m} \right) * e^{(([S]_o - V_m * time) / K_m)}$$

$$y = \left(X2 - P1 * \text{Omega}W \left(\left(\frac{X2}{P1} \right) * \text{EXP} \left(\frac{X2 - P2 * X1}{P1} \right) \right) \right) + P3$$

Integrated Michaelis-Menten minus background, where X1=time, X2=[S]_o, and P1 = K_m, P2 = V_{max}, and P3 = background Bkg.

- VII. The next model (#21) is a two-inhibitor interaction model of Yonetani-Theorell:
Yonetani-Theorell Mutual Exclusivity of Inhibitors

$$y = \frac{V_o}{\left(1.0 + \frac{[I]}{K_i} + \frac{[J]}{K_j} + \frac{[I] * [J]}{(K_i * K_j * \alpha)} \right)}$$

Reference: Yonetani, T. and Theorell, H., Arch. Biochem. Biophys. 106, 243±251 (1964)

- VIII. The next two models (#22, 23) are dose-response (i.e., y response versus x dose) models:
Four-Parameter Logistic equation, Five-Parameter Logistic equation

A general form of the logistic function is the 4/5-Parameter Logistic model:

$$Y = \frac{a - d}{1 + \left(\frac{X}{c} \right)^b} + d$$

where Y is the response and X is the dose variables. The parameter "a" is the response when X = 0, "d" the response at infinite dose, and "c" is the ED₅₀ or IC₅₀ of the dose producing a response halfway between "a" and "d". The slope factor "b" represents the steepness of the dose-response curve in the 5-parameter model.

- IX. The next two models (#24, 25) are typical exponent models:
Descending Exponential equation, Ascending Exponential equation

$$y = (a - b) * e^{(-k * X1)} + b$$

$$y = (a - b) * (1.0 - e^{(-k * X1)}) + b$$

- X. The next model (#26) is a single substrate two-site cooperativity model:
Positive or Negative Substrate Cooperativity

$$y = \frac{V_{max} * \left([S] + \frac{[S]^2}{\alpha * K_m} \right)}{K_m + 2 * [S] + \frac{[S]^2}{\alpha * K_m}}$$

where, $\alpha = 1$ (no cooperativity), $\alpha > 1$ (negative cooperativity), $\alpha < 1$ (positive cooperativity)

Ref. Methods Enzymology 232: 559-576, 597-606, 632-655 (1994)

- XI. The next six models (#27, 28, 29, 30, 31, 32) are Arrhenius temperature versus reaction rate models:

Arrhenius 1st-Order Substance Stability (k), Arrhenius 1st-Order Substance Stability (t90),
Arrhenius Zero-Order Substance Stability (k), Arrhenius Zero-Order Substance Stability (t90),
Arrhenius 2nd-Order Substance Stability (k), Arrhenius 2nd-Order Substance Stability (t90)

Parameters:

k = P1 = /hr (rate of a 10% decrease in physical property at 25 C)

t90 = P1 = hr (time for a 10% decrease in physical property at 25 C)

Ea = P2 = cal/mol (energy of activation)

Co = P3 = uM (initial drug concentration)

Bkg = P4 = baseline

Constants:

RC = 1.9858775 (gas constant)

K1 = 273.15 (0 °C in Kelvin constant)

K2 = 298.15 (25 °C in Kelvin constant)

Variables:

X1 = time (hrs)

X2 = temperature (Celsius)

Zero Order Reaction:

C = Co - k298*time

$$y = P3 - \left((X1 * P1) * e^{\left(\frac{P2}{RC} * \left(\frac{1}{K2} - \frac{1}{X2 + K1} \right) \right)} \right)$$

$$y = P3 - \left(\left(X1 * \left(\frac{\text{LOG}\left(\frac{100}{90}\right)}{P1} \right) \right) * e^{\left(\frac{P2}{RC} * \left(\frac{1}{K2} - \frac{1}{X2 + K1} \right) \right)} \right)$$

1st-Order Reaction minus Baseline:

$$C = C_0 * \exp(-k_{298} * \text{time})$$

$$y = (P3 - P4) * e^{\left(-X1 * P1 * e^{\left(\left(\frac{P2}{RC}\right) * \frac{X2 + K1 - K2}{K2 * (X2 + K1)}\right)}\right)} + P4$$

$$y = (P3 - P4) * e^{\left(-X1 * \left(\frac{\text{LOG}\left(\frac{100}{90}\right)}{P1}\right) * e^{\left(\left(\frac{P2}{RC}\right) * \frac{X2 + K1 - K2}{K2 * (X2 + K1)}\right)}\right)} + P4$$

2nd Order Reaction minus Baseline:

$$1/C = 1/C_0 + k_{298} * \text{time} \rightarrow C = C_0 / (1 + C_0 * k_{298} * \text{time})$$

$$y = \frac{P3 - P4}{1.0 + (P3 - P4) * (X1 * P1) * e^{\left(\left(\frac{P2}{RC}\right) * \left(\frac{1}{K2} - \frac{1}{X2 + K1}\right)\right)}} + P4$$

$$y = \frac{P3 - P4}{1.0 + (P3 - P4) * \left(X1 * \left(\frac{\text{LOG}\left(\frac{100}{90}\right)}{P1}\right)\right) * e^{\left(\left(\frac{P2}{RC}\right) * \left(\frac{1}{K2} - \frac{1}{X2 + K1}\right)\right)}} + P4$$

1. Reference: Incorporating batch effects in the estimation of drug stability parameters using an Arrhenius model, Intel. J. Pharmaceutics 184:165-172 (1999)

XII. The next four models (#33, 34, 35, 36) are integrated forms of Michaelis-Menten Inhibition Models:

Integrated M-M Competitive Inhibition, Integrated M-M Uncompetitive Inhibition, Integrated M-M Noncompetitive Inhibition, Integrated Michaelis-Menten Mixed Inhibition.

e.g., Integrated Mixed Noncompetitive Inhibition Progress of Reaction,

$$\frac{dp}{dt} = \frac{V_{max} * ([S]_0 - [S]_t)}{K_m * \left(1 + \frac{[I]}{K_{i1}}\right) + ([S]_0 - [S]_t) * \left(1 + \frac{[I]}{K_{i2}}\right)}$$

$$y = [P]_t = [S]_0 - [S]_t$$

$$y = [S]_0 - K_{app} * \text{OmegaW} \left(\left(\frac{[S]_0}{K_{app}} \right) * \text{EXP} \left(\frac{[S]_0 - V_{app} * \text{time}}{K_{app}} \right) \right)$$

$$K_{app} = K_m * \frac{\left(1 + \frac{[I]}{K_{i1}}\right)}{\left(1 + \frac{[I]}{K_{i2}}\right)}$$

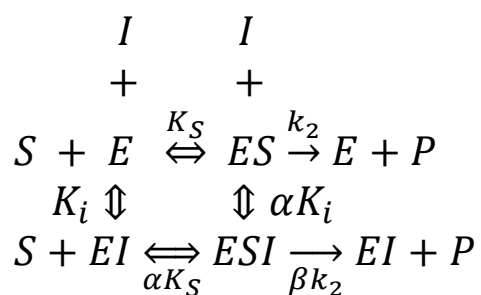
$$V_{app} = \frac{V_{max}}{1 + \frac{[I]}{K_{i2}}}$$

Where the data file contains, X1=time, X2=[I], X3=[So]

- XIII. The next model (#37) is the Botts-Morales General Modifier Model:
Reaction scheme of the General Modifier Mechanism of Botts and Morales.

Scheme for the General Modifier Mechanism.

S: substrate, I: inhibitor, P: product of reaction, K_s: substrate dissociation constant, K_i: inhibitor binding dissociation constant, k₂: catalytic rate constant, and α and β are dimensionless constants that define the type of the inhibition mechanism.



This scheme is based on the rapid-equilibrium assumption and describes the basic mechanism for the all classical reversible inhibition mechanisms. At saturating inhibitor concentrations, the enzyme activity is either fully or partially inhibited depending on the value of β. The inhibition response can be either linear (β = 0) or partial and hyperbolic (0 < β < 1). In other words, at saturating inhibitor concentrations the remaining enzyme activity is either fully or partially decreased, respectively. If β = 1 then both ES and ESI are equally catalytically activity. In contrast, when β = 0 then only the ES complex forms product.

The factor α represents the difference in binding affinities between the substrate binding to the EI complex and the inhibitor binding to the ES complex. When α = 1 the binding is equal, but when α = 0, inhibitor only binds to the free enzyme.

Differing values of α and β can be used to obtain all the common types of inhibition models. Competitive, uncompetitive, noncompetitive, or mixed type inhibition can generally be distinguished by the values of α and K_i.

Values of alpha (a) and beta (b) determine the inhibition type as shown below:

Linear Competitive:	$a = \text{Inf}, b = 0$
Linear Uncompetitive:	$K_i \gg a * K_i, b = 0$
Linear Noncompetitive:	$a = 1, b = 0$
Linear Mixed Noncompetitive:	$1 < a < \text{Inf}, b = 0$
Hyperbolic Competitive:	$1 < a < \text{Inf}, b = 1$
Hyperbolic Uncompetitive:	$0 < a < 1, 0 < b < 1, a = b$
Hyperbolic Noncompetitive:	$a = 1, 0 < b < 1$
Hyperbolic Mixed Noncompetitive:	$1 < a < \text{Inf}, 0 < b < 1$

$$v = \frac{V_m * [S] * \left(\frac{1 + \beta * \frac{[I]}{\alpha * K_i}}{1 + \frac{[I]}{\alpha * K_i}} \right)}{K_s * \frac{\left(1 + \frac{[I]}{K_i} \right)}{\left(1 + \frac{[I]}{\alpha * K_i} \right)} + [S]}$$

Data file: Ks=P1, Vm=P2, Ki=P3, α=P4, β=P5, [S]=X1, [I]=X2

References:

1. Kinetics of Enzyme-Modifier Interactions, Antonio Baici, Springer-Verlag Wien (2015)
2. Botts, J. & Morales, M., Trans. Faraday Soc. 49, 697-707 (1953)
3. The specific velocity plot. A graphical method for determining inhibition parameters for both linear and hyperbolic enzyme inhibitors, Baici, A., Eur. J. Biochem. 119, 9–14 (1981)

- XIV. The next three models (#38, 39, 40) are inhibitor tight-binding models:
 Baici Tight-binding Hyperbolic Noncompetitive Model, Baici Tight-binding Hyperbolic Competitive Model, Baici Tight-binding Modifier 6-parameter Model

Baici-Szedlacsek Hyperbolic Tight-binding Inhibition Models:
 Values of alpha and beta determine the inhibition type.

Hyperbolic Competitive:	alpha > 0, beta = 1
Hyperbolic Uncompetitive:	alpha < 1, beta < 1, alpha = beta
Hyperbolic Noncompetitive:	alpha = 1, beta < 1, alpha > beta
Hyperbolic Mixed Noncompetitive:	alpha > 1, beta < 1, alpha > beta
Linear Inhibition occurs when:	beta = 0

Model 38 - Baici-Szedlacsek Hyperbolic-Noncompetitive Tight-binding Inhibit
 Fix alpha=1 and restricting beta (0 < beta < 1) values.
 (Note: Non-competitive tight-binding is not a function of sigma = So/Km)

Model 39 - Baici-Szedlacsek Hyperbolic-Competitive Tight-binding Inhibition
 Fix beta=1 and restricting alpha (0 < alpha < infinity) values.
 (Note: Competitive tight-binding is a function of sigma = So/Km)

Where, X1=[I], X2=[E], Vo=rate in absence of inhibitor, Ki=inhibition constant, a= alpha, beta=1, sigma=sigma=So/Km

$$v = v_o * (1 - (1 + \sigma)/(\alpha + \sigma))/2$$

$$* \left(\text{SQRT} \left(\left(\left(\alpha * \frac{K_i}{E} \right) * \frac{1 + \sigma}{\alpha + \sigma} + \frac{I}{E} - 1 \right)^2 + 4 * \left(\alpha * \frac{K_i}{E} \right) * \frac{1 + \sigma}{\alpha + \sigma} \right) \right.$$

$$\left. + \frac{\alpha + \sigma + (1 + \sigma)}{\alpha + \sigma - (1 + \sigma)} - \left(\alpha * \frac{K_i}{E} \right) * \frac{1 + \sigma}{\alpha + \sigma} - \frac{I}{E} \right)$$

Model 40 - Baici-Szedlacsek Six Parameter Hyperbolic Tight-binding Inhibition
 Fix sigma=So/Km and restricting alpha (0 < alpha < infinity) values.
 (Note: Tight-binding is a function of sigma = So/Km)

Where, X1=[I], P1=Ki, P2=Vo, P3=alpha, P4=beta, P5=sigma, P6=[E]t

$$v = (v_o/2) * ((\alpha + \sigma - \beta * (1 + \sigma))/(\alpha + \sigma))$$

$$* \left(\sqrt{\left(\left((1 + \sigma) * \alpha * \frac{K_i}{(\alpha + \sigma) * E_t} \right) + \left(\frac{I}{E_t} \right) - 1 \right)^2 + \left(4 * (1 + \sigma) * \alpha * \frac{K_i}{(\alpha + \sigma) * E_t} \right)} \right)$$

$$+ \left(\frac{\alpha + \sigma + \beta * (1 + \sigma)}{(\alpha + \sigma - \beta * (1 + \sigma))} \right) - \left((1 + \sigma) * \alpha * \frac{(K_i)}{(\alpha + \sigma) * E_t} \right) - \left(\frac{I}{E_t} \right)$$

References:

1. Graphical & statistical analysis of hyperbolic tight-binding inhibition, Antonio Baici, Biochem. J. 244: 793-796 (1987)
2. A re-evaluation of the kinetic equations for hyperbolic tight-binding, S.E. Szedlacsek, V. Ostafe, M. Serban, M.O.Vladi, Biochem.J.254:311-312 (1988)
3. Kinetics of Enzyme-Modifier Interactions, Antonio Baici, Springer-Verlag GmbH Wien (2015)

- XV. The next model (#41) is the Gaddum-Schild Logistic Agonist/Antagonist Model:
Gaddum-Schild Logistic Agonist/Antagonist Model

Schild analysis is used to determine the nature of antagonists to its receptor. Schild plots also give information on the potency of competitive antagonists and can hint at the presence of multiple binding sites. Information on whether the antagonist molecule binds with some form of cooperativity can also be inferred. When an antagonist competitive inhibitor competes for agonist binding to its receptor, the dose-response curve shifts to the right without changing the maximum response. The affinity of the competitive inhibitor can be determined by curve-fitting multiple curves each using higher concentrations of inhibitor.

The logarithmic measure of the activity of an antagonist as a log equilibrium constant, pA₂ is the negative log of the concentration of antagonist which would produce a 2-fold shift in the concentration-response curve of an agonist and is directly proportional to the free energy of binding. If the slope of the Schild plot is not exactly 1, but does not differ significantly from it, it is usually preferred to constrain the slope of the line to 1 in calculating an estimated pA₂. The slope of a Schild plot should equal 1 if all of the assumptions underlying the method of analysis are fulfilled. A slope which is significantly greater than 1 may indicate positive cooperativity in the binding of the antagonist, depletion of a potent antagonist from the medium by receptor binding, non-specific binding, or lack of antagonist equilibrium. A slope which is significantly less than 1 may indicate negative cooperativity in the binding, removal of agonist by a saturable uptake process, or it may arise because the agonist is acting at a second receptor type

producing curved Schild plots. The dissociation equilibrium constant K_b for a competitive antagonist is the concentration which would occupy 50% of the receptors at equilibrium. The reciprocal is called the affinity constant or the association constant.

Logistic-Gaddum-Schild Equation:

$$y = \frac{Max - Min}{1 + 10^{\left(\log_{10} \left(10^{\log EC_{50}} * \left(1 + \left(\frac{Antag}{10^{-pA2}} \right)^{SchildSlope} \right) \right) - Agonist \right) * HillSlope}} + Min}$$

Where, $X1 = \log_{10}[\text{agonist}]$ molar and $X2 = [\text{antagonist}]$ molar.

$P1 = \log_{10}(EC_{50})$ molar

$P2 = pA2 = -\log_{10}(\text{antagonist})$ that shifts EC_{50} by factor of 2

$P3 =$ Minimum response plateau

$P4 =$ Maximum response plateau

$P5 =$ Hill slope of the response curve

$P6 =$ Schild slope (equals 1.0 for competitive antagonist)

$[X1]$ and $[X2]$ are the molar concentration of agonist and antagonist, respectively;

$X1 = \log_{10}[\text{agonist}]$ molar

$X2 = [\text{antagonist}]$ molar

$Y =$ dose-response of dependent variable

$\log EC_{50} = \log_{10}(EC_{50})$

$EC_{50} = 10^{(\log EC_{50})}$

$Antag = (1 + (X2 / (10^{(-pA2)}))^{SchildSlope})$

$\log EC = \log_{10}(EC_{50} * Antag)$

$K_b =$ dissociation equilibrium constant for a competitive antagonist

$K_b \text{ of antagonist} = 10^{(-P2)} = 10^{(-pA2)} = 10^{(-\log_{10}(\text{antagonist}))}$

References:

1. Clark, A.J. and Raventos, J. (1937) The antagonism of acetylcholine and of quaternary ammonium salts Quart. J. Exp. Physiol., 26, 375-392.
2. Schild, H.O. (1947) pA , a new scale for the measurement of drug antagonism. Br. J. Pharmacol., 2, 189-206.
3. Gaddum, J.H. (1937) The quantitative effects of antagonistic drugs. J. Physiol., 89, 7P-9P.
4. GADDUM JH. (1957) Theories of drug antagonism. Pharmacol Rev. 9(2), 211-218.
5. Colquhoun, D.. Why the Schild method is better than Schild realised. Trends Pharmacol Sci (2007) vol. 28 (12) pp. 608-14

- XVI. The next models (#42-46) are the Birrell-Yoshino Hyperbolic Substrate Inhibition Model:
Botts-Morales Hyperbolic Substrate Inhibition Model

$$v = \frac{V_m * [S]_o * \left(\frac{1 + \beta * \frac{[S]_o}{\alpha * K_{si}}}{1 + \frac{[S]_o}{\alpha * K_{si}}} \right)}{K_s * \frac{\left(1 + \frac{[S]_o}{K_{si}} \right)}{\left(1 + \frac{[S]_o}{\alpha * K_{si}} \right)} + [S]_o}$$

Models #44-46 are the competitive, uncompetitive, and noncompetitive versions of this model.

Note: It is nearly impossible to obtain reliable K_{si} values using this model unless the V_{max} is already known and masked in the model (fixed to a constant value).

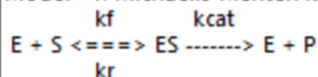
References:

1. Allosteric interactions within subsites of a monomeric enzyme: Kinetics of fluorogenic substrates of PI-specific phospholipase C, Birrell, G.B. et al, Biophys. J. 84, 3264-3275 (2003)
2. Analytical description of the effects of modifiers and of enzyme multivalency upon the steady state catalyzed reaction rate, Botts, J., and Morales, M., Trans. Faraday Soc. 49, 696-707 (1953)
3. Analysis of the substrate inhibition of complete and partial types, Yoshino, M., and Murakami, K., Springerplus 4, 292 (2015)
4. Kinetics of Enzyme-Modifier Interactions, Antonio Baici, Springer-Verlag GmbH Wien (2015)
<https://www.enzyme-modifier.ch/inhibition-and-nonessential-activation>

ODE System of Library Kinetic Models for Simulation:

There are 5 systems of ordinary differential equations models to choose from for simulating.

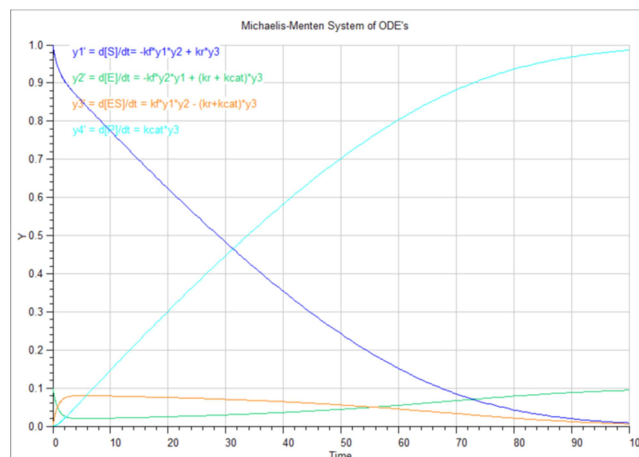
Model #1: Michaelis-Menten Kinetics



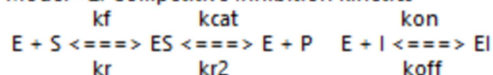
$k_1 = kf, k_2 = kr, k_3 = kcat$

$S = [\text{Substrate}], E = [\text{Enzyme}], P = [\text{Product}]$

$$\begin{aligned} y(1) &= dS/dt = -kf \cdot S \cdot E + kr \cdot ES \\ &= -kf \cdot y(1) \cdot y(1) + kr \cdot y(3) \\ y(2) &= dE/dt = -k_1 \cdot S \cdot E + (kr + kcat) \cdot ES \\ &= -kf \cdot y(1) \cdot y(2) + (kr + kcat) \cdot y(3) \\ y(3) &= dES/dt = kf \cdot S \cdot E - (kr + kcat) \cdot ES \\ &= kf \cdot y(1) \cdot y(2) - (kr + kcat) \cdot y(3) \\ y(4) &= dP/dt = kcat \cdot ES \\ &= kcat \cdot y(3) \end{aligned}$$



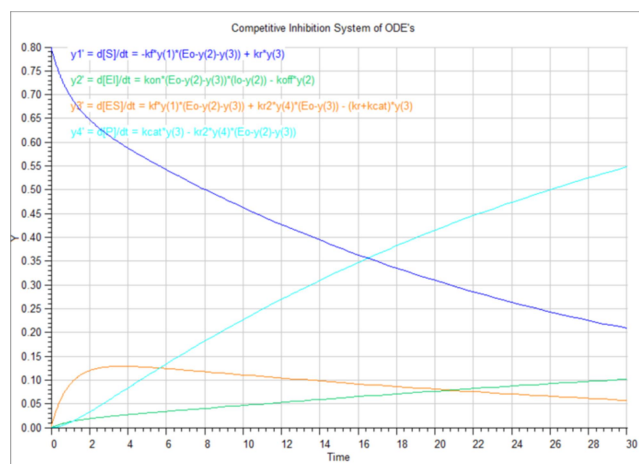
Model #2: Competitive Inhibition Kinetics



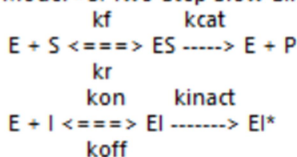
$k_1 = kf, k_2 = kr, k_3 = kcat, k_4 = kr2, k_5 = kon, k_6 = koff$

$S = [\text{Substrate}], Eo = [\text{Enzyme}], Io = [\text{Inhibitor}]$

$$\begin{aligned} y(1) &= dS/dt = -kf \cdot S \cdot (Eo - EI - ES) + kr \cdot ES \\ &= -kf \cdot y(1) \cdot (Eo - y(2) - y(3)) + kr \cdot y(3) \\ y(2) &= dEI/dt = kon \cdot (Eo - EI - ES) \cdot (Io - EI) - koff \cdot EI \\ &= kon \cdot (Eo - y(2) - y(3)) \cdot (Io - y(2)) - koff \cdot y(2) \\ y(3) &= dES/dt = kf \cdot S \cdot (Eo - EI - ES) + kr2 \cdot P \cdot (Eo - ES) - (kr + kcat) \cdot ES \\ &= kf \cdot y(1) \cdot (Eo - y(2) - y(3)) + kr2 \cdot y(4) \cdot (Eo - y(3)) - (kr + kcat) \cdot y(3) \\ y(4) &= dP/dt = kcat \cdot ES - kr2 \cdot P \cdot (Eo - EI - ES) \\ &= kcat \cdot y(3) - kr2 \cdot y(4) \cdot (Eo - y(2) - y(3)) \end{aligned}$$



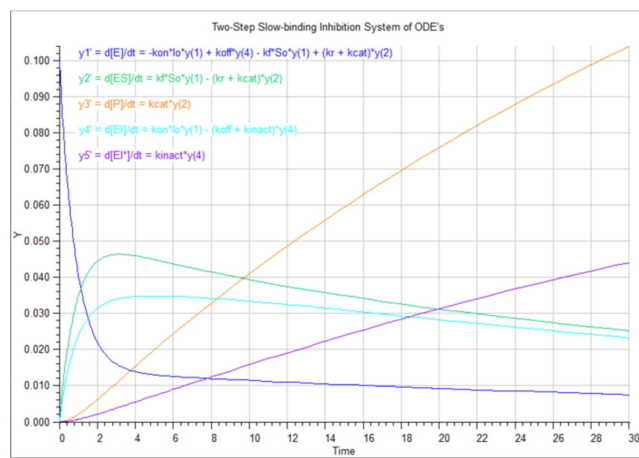
Model #3: Two-Step Slow-Binding Inhibition Kinetics

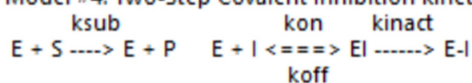


$k_1 = kf, k_2 = kr, k_3 = kcat, k_4 = kon, k_5 = koff, k_6 = kinact$

$S = [\text{Substrate}], E = [\text{Enzyme}], I = [\text{Inhibitor}], P = [\text{Product}]$

$$\begin{aligned} y(1) &= dE/dt = -kon \cdot I \cdot E + koff \cdot EI - kf \cdot S \cdot E + (kr + kcat) \cdot ES \\ &= -kon \cdot Io \cdot y(1) + koff \cdot y(4) - kf \cdot So \cdot y(1) + (kr + kcat) \cdot y(2) \\ y(2) &= dES/dt = +kf \cdot S \cdot E - (kr + kcat) \cdot ES \\ &= kf \cdot So \cdot y(1) - (kr + kcat) \cdot y(2) \\ y(3) &= dP/dt = +kcat \cdot ES \\ &= kcat \cdot y(2) \\ y(4) &= dEI/dt = +kon \cdot I \cdot E - (koff + kinact) \cdot EI \\ &= kon \cdot Io \cdot y(1) - (koff + kinact) \cdot y(4) \\ y(5) &= dEI^*/dt = +kinact \cdot EI \\ &= kinact \cdot y(4) \end{aligned}$$

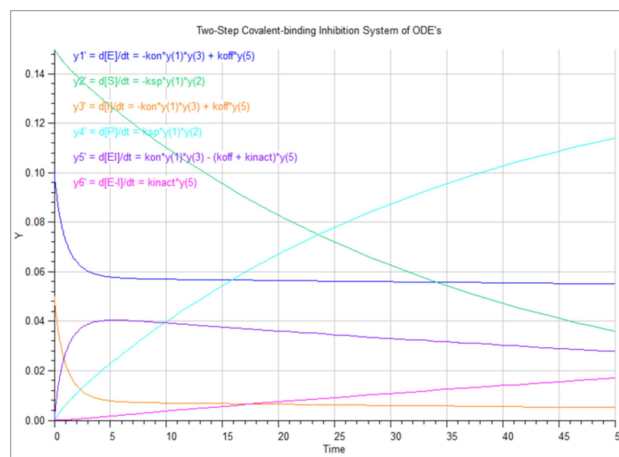
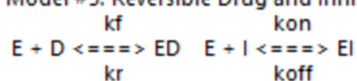


Model #4: Two-Step Covalent Inhibition Kinetics

$k_1 = k_{\text{sub}}, k_5 = k_{\text{on}}, k_6 = k_{\text{off}}, k_7 = k_{\text{inact}}$
 $\text{S} = [\text{Substrate}], \text{E} = [\text{Enzyme}], \text{I} = [\text{Inhibitor}], \text{P} = [\text{Product}]$

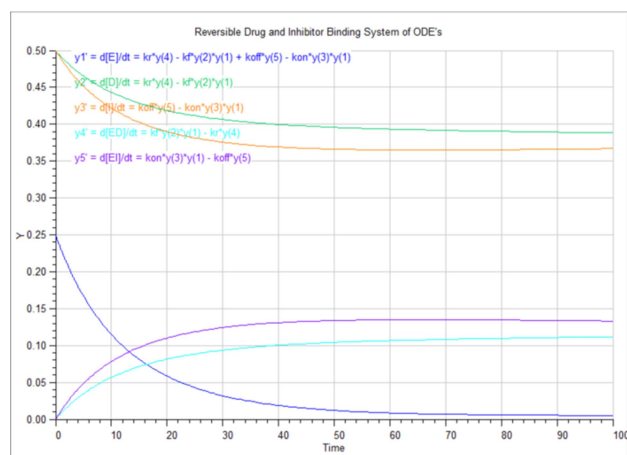
Assumptions: Substrate $[\text{S}]_0 \ll K_m$, Inhibitor $[\text{I}]_0 \sim [\text{E}]_0$,
 Specificity number $k_{\text{sub}} = k_{\text{cat}} / K_m$

$$\begin{aligned}
 y(1) &= dE/dt = -k_{\text{on}}I^*E + k_{\text{off}}*EI \\
 &= -k_{\text{on}}*y(1)*y(3) + k_{\text{off}}*y(5) \\
 y(2) &= dS/dt = -k_{\text{sub}}*S^*E \\
 &= -k_{\text{sub}}*y(2)*y(1) \\
 y(3) &= dI/dt = -k_{\text{on}}I^*E + k_{\text{off}}*EI \\
 &= -k_{\text{on}}*y(3)*y(1) + k_{\text{off}}*y(5) \\
 y(4) &= dP/dt = +k_{\text{sub}}*S^*E \\
 &= k_{\text{sub}}*y(2)*y(1) \\
 y(5) &= dEI/dt = +k_{\text{on}}I^*E - (k_{\text{off}} + k_{\text{inact}})*EI \\
 &= k_{\text{on}}*y(3)*y(1) - (k_{\text{off}} + k_{\text{inact}})*y(5) \\
 y(6) &= dE-I/dt = +k_{\text{inact}}*EI \\
 &= k_{\text{inact}}*y(5)
 \end{aligned}$$

**Model #5: Reversible Drug and Inhibitor Competitive binding**

$k_1 = k_f, k_2 = k_r, k_5 = k_{\text{on}}, k_6 = k_{\text{off}}$
 $\text{D} = [\text{Drug}], \text{E} = [\text{Enzyme}], \text{I} = [\text{Inhibitor}]$

$$\begin{aligned}
 y(1) &= dE/dt = +k_r*ED - k_f*D^*E + k_{\text{off}}*EI - k_{\text{on}}*I^*E \\
 &= k_r*y(4) - k_f*y(2)*y(1) + k_{\text{off}}*y(5) - k_{\text{on}}*y(3)*y(1) \\
 y(2) &= dD/dt = +k_r*ED - k_f*D^*E \\
 &= k_r*y(4) - k_f*y(2)*y(1) \\
 y(3) &= dI/dt = +k_{\text{off}}*EI - k_{\text{on}}*I^*E \\
 &= k_{\text{off}}*y(5) - k_{\text{on}}*y(3)*y(1) \\
 y(4) &= dED/dt = +k_f*D^*E - k_r*ED \\
 &= k_f*y(2)*y(1) - k_r*y(4) \\
 y(5) &= dEI/dt = k_{\text{on}}*I^*E - k_{\text{off}}*EI \\
 &= k_{\text{on}}*y(3)*y(1) - k_{\text{off}}*y(5)
 \end{aligned}$$



How to create a text data file for input to BestCurvFit**Example #1 input data file format: Michaelis-Menten1.dat**

$$y = \frac{(V_{max} * [S])}{(K_m + [S])} = \frac{(P2 * X1)}{(P1 + X1)}$$

where X1=[Substrate]

Data file format:

The first line of the data file contains the intrinsic library model function number (MODEL #).

The model contains two adjustable parameters (P1 and P2).

The lower and upper parameter limits are set to 1.E-06 and 1.E+06, respectively.

None of the parameter values are held constant (Unmask = TRUE).

There is only one curve listed in columns as X1 and Y values.

The one independent variables (X1) is listed first followed by the Y values to fit.

The last line of the data file must contain the word END to finish.

```

Model #1      !<-- Library Model function
10.           100. !<-- Initial parameter values
1.E-06        1.E-06 !<-- Lower limits of parameters
1.E+06        1.E+06 !<-- Upper limits of parameters
TRUE         TRUE   !<-- Masked parameters = FALSE
5    3.2         !<-- Data in columns: X1, Y
10   15.9        !<-- Curve #1
15   35.8
20   45.1
25   69.1
30   75.6
35   76.6
40   83.7
45   80.5
50   90.0
55   87.2
60   94.3
65   99.0
70   90.7
75   88.6
80   97.0
85   101.9
90   90.8
95   90.5
100  105.0
END           !<-- Enter END to exit

```

Example #2 input data file format (User-defined model): Michaelis-Menten.dat

Data file format:

The first line of the data file always contains the model function,

$$y = \frac{(V_{max} * [S])}{(K_m + [S])} = \frac{(P2 * X1)}{(P1 + X1)}$$

where X1=[Substrate]

The model contains two adjustable parameters (P1 and P2).

The lower and upper parameter limits are set to 1.E-06 and 1.E+06, respectively.

None of the parameter values are held constant (Unmask = TRUE).

There is only one curve listed in two columns as X1 and Y values.

The one independent variables (X1) is listed first followed by the Y values to fit.

The last line of the data file must contain the word END to finish.

```

y = P2*X1/(P1 + X1)      !<-- User-defined function (UDF)
10.      100.             !<-- Initial parameter values
1.E-06   1.E-06          !<-- Lower limits of parameters
1.E+06   1.E+06          !<-- Upper limits of parameters
TRUE     TRUE            !<-- Unmasked parameters = TRUE
5    3.2                 !<-- Data in columns: X1, Y
10   15.9                !<-- Curve #1
15   35.8
20   45.1
25   69.1
30   75.6
35   76.6
40   83.7
45   80.5
50   90.0
55   87.2
60   94.3
65   99.0
70   90.7
75   88.6
80   97.0
85   101.9
90   90.8
95   90.5
100  105.0
END                        !<-- Enter END to exit

```

Example #3 input data file format for Noncompetitive Inhibition model #5

Data file format:

We'll use the intrinsic library Model #5 here instead of a UDF.

If you were to use a UDF it would look like,

$$y = \frac{V_{max}}{\left(1.0 + \frac{K_m}{[S]}\right) * \left(1.0 + \frac{[I]}{K_i}\right)} = \frac{P2}{\left(1.0 + \frac{P1}{X1}\right) * \left(1.0 + \frac{X2}{P3}\right)}$$

where X1=[Substrate], X2=[Inhibitor]

The first line of the data file always contains the model function (e.g., Model #5).

The model contains three adjustable parameters (P1, P2 and P3).

The lower and upper parameter limits are set to 1.E-06 and 1.E+06, respectively.

None of the parameter values are held constant (Unmask = TRUE).

There are four curves listed in columns as X1, X2, and Y values.

The variables X1 = [Substrate] and X2 = [Inhibitor].

The two independent variables (X1 and X2) are listed first followed by the Y values to fit.

The last line of the data file must contain the word END to finish.

Model #5			!<-- Library Model function
1.	1.	1.	!<-- Initial parameter estimates
1.E-06	1.E-06	1.E-06	!<-- Lower limits of parameters
1.E06	1.E06	1.E06	!<-- Upper limits of parameters
TRUE	TRUE	TRUE	!<--Unmasked parameters=TRUE or FALSE
50	0	0.032	!<-- Data in columns: X1, X2, and Y of Curve #1
100	0	0.055	
200	0	0.080	
250	0	0.090	
333	0	0.102	
500	0	0.116	
700	0	0.130	
50	100	0.017	!<-- Curve #2 (X2 value changed)
100	100	0.028	
200	100	0.045	
250	100	0.050	
333	100	0.065	
500	100	0.072	
700	100	0.078	
50	200	0.010	!<-- Curve #3 (X2 value changed)
100	200	0.018	
200	200	0.030	

250	200	0.036	
333	200	0.041	
500	200	0.048	
700	200	0.052	
50	350	0.006	!<-- Curve #4 (X2 value changed)
100	350	0.012	
200	350	0.019	
250	350	0.023	
333	350	0.027	
500	350	0.030	
700	350	0.035	
END			!<-- Enter END to exit

Example #4 input data file format for Yonetani-Theorell exclusivity of inhibitor interactions with an enzyme: Model #21,

where X1=[inhibitor I], X2=[inhibitor J], y=initial enzyme velocity in the presence of inhibitors I and J, P1=dissociation constant K_i , P2=dissociation constant K_j , P3=interaction term α , and P4=uninhibited rate V_o .

$$y = \frac{V_o}{\left(1.0 + \frac{[I]}{K_i} + \frac{[J]}{K_j} + \frac{[I] * [J]}{(K_i * K_j * \alpha)}\right)} = \frac{P4}{\left(1.0 + \frac{X1}{P1} + \frac{X2}{P2} + \frac{X1 * X2}{(P1 * P2 * P3)}\right)}$$

where X1=[Inhibitor 1], X2=[Inhibitor 2]

The method involves measuring the initial velocity of the enzyme at different concentrations of two inhibitors. The effects of the two inhibitors on the velocity of the enzymatic reaction where V_o is the initial enzyme velocity in the absence of inhibitors, y is the initial velocity in the presence of both inhibitors, K_i and K_j are the dissociation constants for inhibitors I and J, respectively, and α is an interaction term that defines the effect of the binding of one inhibitor on the affinity of the second inhibitor. If the two inhibitors bind in a mutually exclusive fashion, $\alpha = \text{infinity}$. If the two bind completely independently, $\alpha = 1$. If the two inhibitors bind nonexclusively but influence each other's affinity for the enzyme, then α will be finite, but less than or greater than 1. When α is less than 1, the binding of one inhibitor increases the affinity of the enzyme for the second inhibitor, and the binding of the two is said to be synergistic (i.e., exhibiting positive cooperativity). When α is greater than 1, the binding of one inhibitor decreases the affinity of the enzyme for the second inhibitor, and in this case the binding is said to be antagonistic (i.e., exhibiting negative cooperativity).

1. Reference: Yonetani, T. and Theorell, H., Arch. Biochem. Biophys. 106, 243±251 (1964)

Data file format:

We'll use the intrinsic library Model #21

The first line of the data file always contains the model function (e.g., Model #21).

The model contains 4 adjustable parameters (P1, P2, P3, and P4).

The lower and upper parameter limits are set to 1.E-06 and 1.E+03, respectively.

None of the parameter values are held constant (Unmask = TRUE).

There are 5 curves listed in columns as X1, X2, and Y values.

The variables X1 = [Inhibitor I] and X2 = [Inhibitor J].

The 2nd variable X2 is always used to determine the number of curves in the data set.

The two independent variables (X1 and X2) are listed first followed by the Y values to fit.

The last line of the data file must contain the word END to finish.

```

MODEL #21                                !<-- Library Model function
1.          1.          1.          1.    !<-- Initial parameter estimates
1.E-06      1.E-06      1.E-06      1.E-06 !<-- Lower limits of parameters
1.E03       1.E03       1.E03       1.E03 !<-- Upper limits of parameters
T           T           T           T     !<-- UnMasked parameters = T
1.500       1.500       74.683    !<-- Data in columns: X1, X2, and Y of Curve #1
3.750       1.500       71.556
7.125       1.500       67.329
12.188      1.500       61.848
19.781      1.500       55.117
31.172      1.500       47.383
48.258      1.500       39.143
73.887      1.500       31.046
112.330     1.500       23.693
169.995     1.500       17.483
1.500       7.125       40.036    !<-- Curve #2 (X2 value changed)
3.750       7.125       38.360
7.125       7.125       36.094
12.188      7.125       33.155
19.781      7.125       29.547
31.172      7.125       25.401
48.258      7.125       20.984
73.887      7.125       16.643
112.330     7.125       12.702
169.995     7.125       9.372
1.500       19.780      19.590    !<-- Curve #3 (X2 value changed)
3.750       19.780      18.770
7.125       19.780      17.661
12.188      19.780      16.223
19.781      19.780      14.458
31.172      19.780      12.429
48.258      19.780      10.268
73.887      19.780      8.144
112.330     19.780      6.215
169.995     19.780      4.586

```

1.500	48.250	9.116	!<-- Curve #4 (X2 value changed)
3.750	48.250	8.735	
7.125	48.250	8.219	
12.188	48.250	7.549	
19.781	48.250	6.728	
31.172	48.250	5.784	
48.258	48.250	4.778	
73.887	48.250	3.790	
112.330	48.250	2.892	
169.995	48.250	2.134	
1.500	112.330	4.137	!<-- Curve #5 (X2 value changed)
3.750	112.330	3.964	
7.125	112.330	3.730	
12.188	112.330	3.426	
19.781	112.330	3.053	
31.172	112.330	2.625	
48.258	112.330	2.169	
73.887	112.330	1.720	
112.330	112.330	1.313	
169.995	112.330	0.969	
END			!Enter 'END' to exit run

Example #5 input data file format for Incorporating batch effects in the estimation of drug stability parameters using an Arrhenius model # 28

1. Reference: Intel. J. Pharmaceutics 184:165-172 (1999)

Arrhenius 1st-Order Reaction Stability, where X1=[time], X2=[temperature (°C)]

$$y = (C_o - Bkg) * e^{-\left(time * \left(\frac{LOG(\frac{1.}{0.9})}{t_{90}} \right) * e^{\left(\left(\frac{E_a}{1.9859} \right) * \left(\frac{1.}{298.15} - \frac{1}{temperature + 273.15} \right) \right)} \right)} + Bkg$$

$$y = (P3 - P4) * e^{-\left(X1 * \left(\frac{LOG(\frac{1.}{0.9})}{P1} \right) * e^{\left(\left(\frac{P2}{1.9859} \right) * \left(\frac{1.}{298.15} - \frac{1}{X2 + 273.15} \right) \right)} \right)} + P4$$

Where,

P1 = t90 = time (hr) for a 10% decrease in physical property at 25 °C

P2 = Ea = energy of activation (cal/mol)

P3 = Co = initial drug concentration (μM)

P4 = baseline (background signal)

1st-Order Reaction minus Baseline:

Initial Values:

P1 = t90 = 1.E00 hr (time for a 10% decrease in physical property at 25 C)

P2 = Ea = 1.E03 cal/mol (energy of activation)

P3 = Co = 1.E01 uM (initial drug concentration)

P4 = baseline= 10.0

Final Values:

P1 = t90 = 5.54 hr (time for a 10% decrease in physical property at 25 C)

P2 = Ea = 14241. cal/mol (energy of activation)

P3 = Co = 57.0 uM (initial drug concentration)

P4 = baseline= 10.0

Data file format:

We'll use the intrinsic library Model #28

The first line of the data file always contains the model function (e.g., Model #28).

The model contains 4 adjustable parameters (P1, P2, P3, and P4).

The lower and upper parameter limits are set to 1.E-06 and 1.E+03, respectively.

None of the parameter values are held constant (Unmask = TRUE).

There are 4 curves listed in columns as X1, X2, and Y values.

The variables X1 = [time] and X2 =[temperature (°C)].

The 2nd variable X2 is always used to determine the number of curves in the data set.

The two independent variables (X1 and X2) are listed first followed by the Y values to fit.

The last line of the data file must contain the word END to finish.

```

MODEL #28                                !<-- Library Model function
1.          1.E03          10.          10.    !<-- Initial parameter estimates
1.E-06      1.E-06      1.E-06      1.E-06 !<-- Lower limits of parameters
1.E03      1.E03      1.E03      1.E03 !<-- Upper limits of parameters
T          T          T          T      !<-- UnMasked parameters = T
0.1  60  55.89481729          !<-- Data in columns: X1, X2, and Y of Curve #1
1    60  47.0472291
2    60  39.20206775
2.5  60  35.92641932
3    60  33.01820626
5    60  24.30168551
6.5  60  20.00861615
0.1  55  56.20118813          !<-- Curve #2 (X2 value changed)
1    55  49.59594576
2    55  43.35827491
5    55  29.94629196

```

6	55	26.80409137	
11.7	55	16.32508479	
0.1	45	56.59635391	!<-- Curve #3 (X2 value changed)
2	45	49.55299761	
4	45	43.28594936	
6	45	38.01189523	
9.2	45	31.25558027	
21	45	17.68164233	
26	45	14.99065955	
0.1	35	56.80531963	!<-- Curve #4 (X2 value changed)
4	35	49.80999782	
9	35	42.3489377	
24	35	27.35655916	
29	35	24.10364938	
34	35	21.46038936	
50	35	15.898975	
120	35	10.32279495	
END			! Enter 'END' to exit run

Example #6 input data file format for Rapid Equilibrium Random Bi-Bi model # 12

A random order two-substrate, two-product (Bi-Bi) mechanism is one in which any substrate (i.e., [A] or [B]) can bind to the enzyme first and any product can leave first. There are two separate binding sites on the enzyme, one for [A]/[AX] and one for [B]/[BX]. It is a sequential enzyme reaction that involves the formation of a ternary enzyme complex (e.g., one enzyme and two substrates, [A] and [B]).

$$y = \frac{V_{max}}{1.0 + \left(\frac{K_b}{[B]}\right) + \left(\frac{K_a}{[A]}\right) + \left(\frac{K_{ia} * K_b}{[A] * [B]}\right)}$$

$$y = \frac{P4}{\left(1.0 + \left(\frac{P2}{X2}\right) + \left(\frac{P1}{X1}\right) + \left(\frac{P3 * P2}{X1 * X2}\right)\right)}$$

where X1=[A], X2=[B]

Data file format:

We'll use the intrinsic library Model #12

The first line of the data file always contains the model function (e.g., Model #12).

The model contains 4 adjustable parameters (P1, P2, P3, and P4).

The lower and upper parameter limits are set to 1.E-04 and 1.E+04, respectively.

None of the parameter values are held constant (Unmask = TRUE).

There are 4 curves listed in columns as X1, X2, and Y values.

The variables X1 = [Substrate A] and X2 = [Substrate B].

The 2nd variable X2 is always used to determine the number of curves in the data set.

The two independent variables (X1 and X2) are listed first followed by the Y values to fit.

The last line of the data file must contain the word END to finish.

```

MODEL #12                                !<-- Library Model function
1.          1.          1.          1.    !<-- Initial parameter estimates
1.E-04      1.E-04      1.E-04      1.E-04 !<-- Lower limits of parameters
1.E04       1.E04       1.E04       1.E04 !<-- Upper limits of parameters
T           T           T           T     !<-- UnMasked parameters = T
0.25 0.25 0.062                                !<-- Data in columns: X1, X2, and Y of Curve #1
0.70 0.25 0.139
1.50 0.25 0.144
4.00 0.25 0.193
0.25 0.40 0.100                                !<-- Curve #2 (X2 value changed)
0.70 0.40 0.162
1.50 0.40 0.212
4.00 0.40 0.239
0.25 0.70 0.141                                !<-- Curve #3 (X2 value changed)
0.70 0.70 0.228
1.50 0.70 0.286
4.00 0.70 0.371
0.25 1.50 0.164                                !<-- Curve #4 (X2 value changed)
0.70 1.50 0.298
1.50 1.50 0.397
4.00 1.50 0.499
0.25 4.00 0.189                                !<-- Curve #5 (X2 value changed)
0.70 4.00 0.358
1.50 4.00 0.498
4.00 4.00 0.658
END      ! END TO EXIT RUN

```

Example #7 input data file format for Integrated Michaelis-Menten Model with background subtraction model # 19

Enzyme + [S] \rightleftharpoons [ES] \rightarrow Enzyme + Product

The change in product P(t) with time t,

y = product $P_t = [S]_o - [S]_t$

$$y = P_t + Bkg = ([S]_o - [S]_t) + Bkg,$$

$$\text{where } [S]_t = K_m * \text{Omega}W(W)$$

$$y = ([S]_o - K_m * \text{Omega}W(W)) + Bkg$$

$$W = \left(\frac{[S]_o}{K_m} \right) * e^{(([S]_o - V_m * \text{time}) / K_m)}$$

$$y = \left(X2 - P1 * \text{Omega}W \left(\left(\frac{X2}{P1} \right) * \text{EXP} \left(\frac{X2 - P2 * X1}{P1} \right) \right) \right) + P3$$

Integrated Michaelis-Menten minus background, where $X1 = \text{time}$, $X2 = [S]_o$, and $P1 = K_m$, $P2 = V_{\max}$, and $P3 = \text{background Bkg}$.

NOTE: The data file requires time, $[S]_o$, and product y data columns.

References:

1. A closed form solution for time-dependent enzyme kinetics
S. Schnell & C. Mendoza, J. Theoretical Biology, 187: 207-212 (1997)
2. Explicit reformulation of time-dependent solution for a Michaelis-Menten enzyme reaction model
M. Golicnik, Analytical Biochemistry, 406: 94-96 (2010)
3. Estimation of Kinetic Parameters for Enzyme Inhibition Reaction Models Using Direct Time-Dependent Equations for Reactant Concentrations
M. Golicnik, Acta Chim. Slov., 59: 207-211 (2012)
4. On the Lambert W Function and Its Utility in Biochemical Kinetics
M. Golicnik, Biochem. Engineering J., 63: 116-123 (2012)
5. Progress curve analysis for enzyme and microbial kinetic reactions using explicit solutions based on the Lambert W function
Goudar, C.T., Harris, S.K., McInerney, M.J., Suflita, J.M., Journal of Microbial Methods, 59: 317-326 (2004)

Data file format:

We'll use the intrinsic library Model #19

The first line of the data file always contains the model function (e.g., Model #19).

The model contains 3 adjustable parameters (P1, P2, and P3).

The lower and upper parameter limits are set to 1.E-06 and 1.E+06, respectively.

None of the parameter values are held constant (Unmask = TRUE).

There is one curve listed in columns as X1, X2, and Y values.

The variables X1 = time and X2 = [S]_o.

The 2nd variable X2 is always used to determine the number of curves in the data set. In this case since it doesn't change, there is only one curve for this model.

The two independent variables (X1 and X2) are listed first followed by the Y values to fit.

The last line of the data file must contain the word END to finish.

```

MODEL #19                                !<-- Library Model function
50.          1.          1.              !<-- Initial parameter estimates
1.E-06       1.E-06     1.E-06          !<-- Lower limits of parameters
1.E06       1.E06     1.E06          !<-- Upper limits of parameters
T           T          T              !<-- UnMasked parameters = T
0.01  537.  0.0          !<-- Data in columns: X1, X2, and Y of Curve #1
10   537.  57.3
20   537.  114.6
30   537.  182.6
40   537.  229.2
50   537.  272.1
60   537.  313.7
70   537.  364.5
80   537.  408.2
90   537.  449.7
100  537.  464.1
110  537.  509.9
120  537.  500.6
130  537.  529.9
140  537.  515.6
150  537.  529.9
160  537.  528.5
170  537.  533.5
180  537.  534.2
190  537.  534.2
200  537.  533.5
END   ! ENTER 'END' TO EXIT RUN

```

Example #8 input data file format for the Five Parameter Logistic Model using a User-defined function (UDF)

The general form of the Five-Parameter Logistic model is:

$$Y = \frac{a - d}{1 + \left(\frac{X}{c}\right)^b} + d$$

where Y is the response and X is the dose variables. The parameter "a" is the response when X = 0, "d" the response at infinite dose, and "c" is the ED₅₀ or IC₅₀ of the dose producing a response halfway between "a" and "d". The slope factor "b" represents the steepness of the dose-response curve.

Let's consider fitting four dose-response data sets (4 curves) to this model.

$$y = \frac{a - d * (a > d)}{1 + \left(\frac{[X]}{c1 * (Index = 1) + c2 * (Index = 2) + c3 * (Index = 3) + c4 * (Index = 4)} \right)^b} + d$$

The UDF to include in the BestCurvFit data file is,

$$y = \frac{P7 - P6 * (P7 > P6)}{1 + \left(\frac{X1}{P1 * (X2 = 1) + P2 * (X2 = 2) + P3 * (X2 = 3) + P4 * (X2 = 4)} \right)^{P5}} + P6$$

where X1 is the dose and X2 is the index of the curve (i. e., 1, 2, 3, and 4).

For the first curve, the Index equals 1, and (X2 = 1) is TRUE or 1.
and the other Indices (X2 = 2), (X2 = 3), (X2 = 4) are FALSE or 0.

NOTE: The data file requires Dose, Index, and y response data columns.

Data file format:

The first line of the data file always contains the UDF model function.

The model contains 3 adjustable parameters (P1, P2, P3, P4, P5, P6, and P7).

The lower and upper parameter limits are set to 1.E-06 and 1.E+06, respectively.

None of the parameter values are held constant (Unmask = TRUE).

There are four curves listed in columns as X1, X2 (curve index), and Y values.

The variables X1 = dose and X2 = index of each curve (e.g., 1, 2, 3, and 4).

The 2nd variable X2 is always used to determine the number of curves in the data set. In this case since there are four curves, X2 = 1, 2, 3, and 4 for each curve, respectively.

The last line of the data file must contain the word END to finish.

BESTCURVFIT

$$Y=(P7-P6*(P7>P6))/(1 + (X1/(P1*(X2=1) + P2*(X2=2) + P3*(X2=3) + P4*(X2=4)))^P5) + P6$$

1.	1.	1.	1.	1.	1.	1.
1.E-06	1.E-06	1.E-06	1.E-06	1.E-06	1.E-06	1.E-06
1.E06	1.E06	1.E06	1.E06	1.E01	1.E06	1.E06
T	T	T	T	T	T	T
10.000	1	85.128				
20.000	1	50.500				
30.000	1	27.360				
40.000	1	15.872				
50.000	1	10.097				
60.000	1	6.968				
70.000	1	5.139				
80.000	1	4.000				
90.000	1	3.252				
100.00	1	2.740				
10.000	2	94.032				
20.000	2	73.640				
30.000	2	50.500				
40.000	2	33.429				
50.000	2	22.587				
60.000	2	15.872				
70.000	2	11.626				
80.000	2	8.849				
90.000	2	6.968				
100.00	2	5.651				
10.000	3	97.000				
20.000	3	85.128				
30.000	3	67.571				
40.000	3	50.500				
50.000	3	37.040				
60.000	3	27.360				
70.000	3	20.599				
80.000	3	15.872				
90.000	3	12.520				
100.00	3	10.097				
10.000	4	98.890				
20.000	4	94.032				
30.000	4	85.128				
40.000	4	73.640				
50.000	4	61.590				
60.000	4	50.500				
70.000	4	41.078				
80.000	4	33.429				

BESTCURVFIT

90.000 4 27.360
 100.00 4 22.587
 END ! END TO EXIT RUN

RESULTS:

SSR value:

1.078805E-06

Model Information Criterion (MIC):

-2.3818E+01

Model:

$$y = (P7 - P6 * (P7 > P6)) / (1 + (X1 / (P1 * (X2 = 1) + P2 * (X2 = 2) + P3 * (X2 = 3) + P4 * (X2 = 4)))^{P5} + P6$$

Final Parameter Values:

P1	P2	P3	P4	P5	P6	P7
20.00	30.00	40.00	60.00	2.500	0.9999	100.0

Parameter Standard Errors:

SE1	SE2	SE3	SE4	SE5	SE6	SE7
4.92E-05	6.40E-05	7.76E-05	1.05E-04	9.73E-06	1.00E-04	1.26E-04

Parameter Significance (p-value < 5.E-02):

p-value1	p-value2	p-value3	p-value4	p-value5	p-value6	p-value7
2.55E-11	2.55E-11	2.55E-11	2.55E-11	2.55E-11	2.55E-11	2.55E-11

Eigenvalues values < 1.52E-06 may suggest parameter redundancy:

1	2	3	4	5	6	7
3.02E+00	2.10E+00	5.86E-01	5.35E-01	5.11E-01	1.60E-01	9.42E-02

Principal Component Parameter Influence (%)

P1	P2	P3	P4	P5	P6	P7
0.05	0.04	0.02	0.00	99.32	0.55	0.03

RUNS STATISTIC: PASS if $|Run - ExpRun| / (StdDev Run) < 2.58$ (pval=1%)

LENGTH	RUNS	Value	RANDOM RUNS
1	13.	-0.424	PASS
2	10.	0.891	PASS
3	2.	-0.485	PASS

Brief Explanation of Terms

Data File Name

The name of the file where the substrate and inhibitor concentration and velocity of reaction data are stored on disk. These file names are appended with the three-letter extension DAT (e.g., filename.dat). The data file is loaded by using the 'File/Open' option. The name of the file can be changed by using the 'File/Save' option in the main menu.

Dissociation Constant Ks

The Ks is a fitted parameter that represents the substrate concentration required to achieve half of the maximum velocity of the enzyme. The smaller the value of the Ks, the greater the affinity of the substrate for the enzymes active site.

Maximum Velocity Vmax

The Vmax is a fitted parameter which represents the maximum velocity of the enzyme when the substrate(s) is at a saturating concentration. The greater the value of the Vmax, the greater is the enzyme-catalyzed reaction velocity.

Slope or Hill coefficient N

The N value represents the change in the slope of the fitted curve.

Inhibition Parameter Ki1

The K1 is equivalent to the Ki, a dissociation constant for the enzyme inhibitor complex.

Constant Parameter Ki2

The K2 is equivalent to the Ki, a dissociation constant for the enzyme inhibitor complex.

Model Information Criterion (MIC)

The MIC is a modification of the Akaike Information Criterion (AIC) test. MIC is a normalized AIC which divides the logarithm of the ratio of the sum-of-squared deviations (SSQ) to the SSQ of Mean deviations by the total number of observations. In addition, the MIC incorporates the negative contributions of outlying data, non-randomness of residuals, and parameter redundancy. The smaller the MIC value, including negative values, the better the fitted curve to the data points. The MIC is helpful in comparing the final least squares curve-fits of two competing models using the same observed data set.

$$MIC = LOG \left(\frac{\sum_{i=1}^n \left(\frac{y(i) - yfit(i)}{sigy(i)} \right)^2}{\sum_{i=1}^n \left(\frac{y(i) - \sum_{j=1}^n y(j)}{n} \right)^2} \right) + \frac{A}{B}$$

n = number of data points

np = number of parameter values

nse = number of significant parameter values

nmsk = number of masked parameters

$A = n + np + nse - nmsk$

$B = n - np - nse - nmsk - 2$

Runs Test

The Runs test determines statistically whether the data is randomly distributed around the fitted curve at the 95 % confidence level. If the model fits the data well, the residuals between the observed velocity values and the fitted curve should have runs of positive and negative residuals which are at random. A failed Runs test may suggest that there is a systematic departure of the data about the fitted curve and that the data may not fit the chosen inhibition model well. At times, however, the Runs test fails when the data still fits the model well. In such instances, the goodness-of-fit value, the number of outlying data, and whether or not the parameters are redundant should also be considered as always.

Standard Error of the Parameters

The standard errors are approximate errors of each fitted kinetic parameter value. These values should generally not exceed a 25% coefficient of variation [$100 \times (SE/parameter) < 25\%$] when the chosen model is a good fit to the data.

BestCurvFit tests the redundancy of the best fitted parameter values by estimating the Student's t value associated with the parameter estimates as, $t = \text{Parameter} / \text{Sqrt}(\text{variance of parameter})$. The t-value is calculated as the ratio of the parameter estimate to its standard error. The standard error is estimated by the square root of the asymptotic variance of the parameter estimate. A high t-value would suggest that the parameter is well determined in the model (reliable). A small t-value indicates that the estimated parameter is poorly determined. This means that the confidence regions (error) of the parameter may include zero.

The calculated t-values in BestCurvFit are compared by reference to the Student's t-distribution with n-p (n data - p parameters) degrees of freedom. If the parameters, using this test, are significantly different from zero at a p-value of 0.05 (95% confidence) they are not considered redundant. If they are not significantly different from zero with 95% confidence then the parameter values are said to be "redundant". If they are redundant, they usually have large standard errors associated with them. The exception is when the model equation has parameters that tend to be highly correlated to each other.

Outlier Values

Enzyme velocity values which depart significantly from the fitted curve are considered influential data points. The modified Thompson Tau test is used to find outliers or influential data values. When the Studentized residual exceeds Tau, the data point is considered an influential data point. Outlying velocity values are identified when the resulting data are displayed from the main menu.

Standard Error of Regression

This value is used as an indicator of the progression of the nonlinear regression routine to fit the data. This value is not an indicator of the goodness-of-fit of the data to the model. The Model Information Criterion (MIC) is, however, a better indicator of the goodness-of-fit of the model to the experimental

data since it takes into consideration not only the SSQ but also the number of parameters (e.g., degrees of freedom of the model), whether or not they are redundant, outlying data, and the randomness of the residuals (e.g., Runs test).

Masked Parameter Values

Individual parameter values (e.g., P1, P2, ...P8) can be masked (e.g., set to FALSE) in the data file to fix their value to a constant in the curve-fitting analyses.

Reasons Behind Weight Functions

There are a number of reasons why a weight function may be used in statistics or scientific applications. This includes: Weighting for accuracy: giving greater weight to more accurate measurements and less weight to measurements known to be less precise. Compensating for bias: giving greater weight to measurements known to be less biased.

Graph of the Data

The data pairs and fitted curve(s) are plotted on a linear-linear or linear-log axis. The X and Y axis labels and the title may be changed using the Labels option on the plot menu. The graph is scaled automatically to the data and the fitted curve. The graph can be printed to a printer using the 'Print' option, or saved to a bmp file using the 'Save' option.

Curve-fit Data and Residuals

The observed and calculated velocity data, the substrate and inhibitor concentrations, and the residual response values are listed. Outlying data are identified next to the velocity values. The data may be printed using the 'Print' option.

Nonlinear Regression Algorithms

BestCurvFit curve-fits experimental substrate, inhibitor, and velocity inhibition data to mathematical models. Nonlinear curve-fitting of the untransformed data to the chosen kinetic model is an improvement over the double reciprocal plot transformation and analysis of the data by linear least squares regression of the transformed data set. Outlying velocity data point can unduly influence the outcome of linear regression analyses when using the Lineweaver-Burk double-reciprocal plot.

BestCurvFit uses several nonlinear regression methods in tandem, the Random Search, Hooke-Reeves, Nelder-Mead Simplex, Quadratic-programming, and the Gauss-Newton method as modified by Marquardt and Levenberg. These routines are implemented based on the concepts found in the numerical algorithms described in Press, W.H., Flannery, B.P., Teukolsky, S.A., and Vetterling, W.T., Numerical recipes in FORTRAN, Cambridge University Press (1992).

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