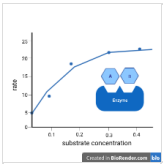

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Fitting enzyme kinetics data with Solver

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1 *Works for me* This protocol may be deleted by the owner

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ABSTRACT

Reaction kinetics are a fundamental component of the biochemical characterization of a biomolecule. The V_{max} and the K_M values derived from experiments describe how fast a biomolecule catalyzes the reaction and the approximate affinity between biomolecule and substrate. For almost a century, these properties have been used to describe the mechanism and functions of enzyme and other biomolecules. Fitting data to calculate kinetic values like the V_{max} and the K_M values is a fundamental skill in biochemistry.

This protocol describes fitting data determined at several substrate concentrations to calculate V_{max} and K_M values using Excel/Google Sheets and the macro with these programs Solver. This protocol is based on the work of Kemmer and Keller.

This protocol is written for students in Biochemistry at James Madison University, however the approach is general enough for any enzyme kinetics data.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Kemmer, G., and Keller, S. (2010) Nonlinear least-squares data fitting in Excel spreadsheets. Nat. Protoc. 5, 267–281.

MATERIALS TEXT

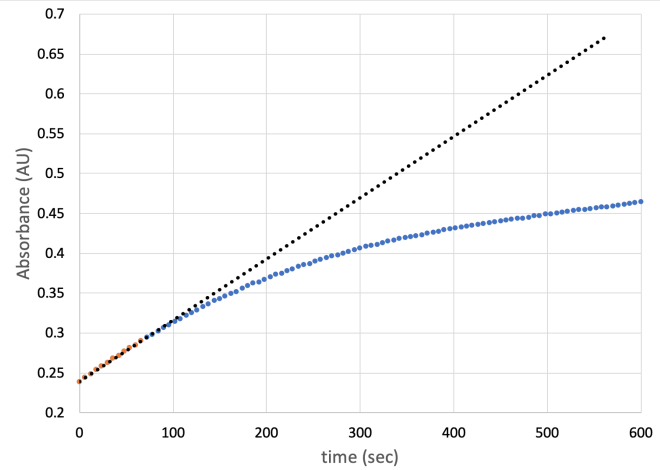
Microsoft Excel or Google Sheets
Kinetics data from at least 5 concentrations of substrate

Calculating intial rates

1 Calculate the initial rate from the slope of the product formed vs. time plot.

It is important to calculate the rate when the plot of product formed vs. time is linear with time. This ensures that the enzyme is in the **steady-state**. Typically this is in the early part of the reaction before more than 10% of the substrate has been converted to product (see figure). *The rate is the slope of the line.*

In the steady-state, the concentration of enzyme bound complexes is not changing with time and the enzyme is catalyzing the reaction at the fastest possible rate at that concentration of substrate.



Example of fitting to calculate the initial velocity. Blue points are data. Blank dashed line shows the fitting of the trendline to the portion of the data where it is linear and presumably the reaction is still in the steady-state. The slope of the black dashed line would be the initial velocity or initial rate.

1.1 Repeat for each concentration of substrate.

1.2 Subtract the rate of the background reaction from each rate.

Background rate is typically from a reaction performed in the absence of enzyme which shows the rate of the reaction in the absence of catalysis.

1.3 Record the rate at each concentration of substrate in the table below. Be sure to modify the units to match the units from the experiment. Add rows to the table as needed.

Concentration of substrate (uM)	Observed Rate (uM/sec)

Plotting data to estimate kinetic values

2 Plot rate (y-axis) vs Substrate concentration (x-axis) and estimate the V_{max} and K_M values using the plot. Plot the data as a scatter plot (no lines)

The V_{max} estimate is the y-value where the graph levels out. The K_M is the x-value at 1/2 Vmax.

2.1 Record your estimates with units of V_{max} and K_M in the table below.

Vmax		
Km		

Fitting data to calculate Vmax and Km

3 To draw a trendline, you'll need to calculate theoretical values for rates using the estimated V_{max} and K_M values from 2.1 and the Michaelis-Menten Equation. This is your V Calculated (V calc). This will also help you calculate the correct Vmax and Km values later on.

4 In Microsoft Excel or Google Sheets, set up a table with headers as shown below:

Concentration of substrate	Observed rate	Theoretical rate (V Calc)	Squared residual		Variables	Estimates	Units
					Vmax		
					Km		
					SSR		

4.1 Input your data table from step 1.3 into columns A and B.

4.2 Input your estimates of V_{max} and K_M from step 2.1 into the appropriate box in column G

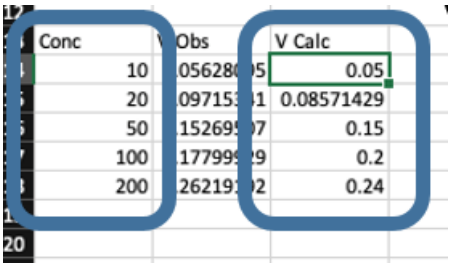
5 In column C, insert the following code to use the Michaelis-Menten equation to calculate the theoretical rate given the estimates for V_{max} and K_M.

```
=($G$2 * A2)/($G$3 + A2)
```

This code uses the Michaelis-Menten equation $v = \frac{V_{max} \times [S]}{K_M + [S]}$ to calculate the rate based on the

estimates. The $ locks those values in so that the equation can be "dragged" down for all concentrations of substrate without needing to re-write the equation.

5.1 "Drag" the equation down until the theoretical rate has been calculated for all the concentrations of substrate. After this step the table will appear similar to below:



5.2 In Column D, insert the following code to calculate the squared residual value.

```
=(B2-C2)^2
```

The squared residual will be used to calculate error and refine the estimates of V_{max} and K_M. It is the squared difference between the real rate and the calculated rate. For a perfect match the squared residual should be zero.

5.3 "Drag" the equation down until the squared residual has been calculated for all the concentrations of substrate.

5.4 In cell G4, insert the following code to calculate the sum of squared residuals. Replace DXX with D followed by the number of the last substrate concentration (e.g. D10 if there are 9 substrate concentrations).

The resulting number is the sum of squared residuals or SSR.

```
=sum(D2:DXX)
```

6 Use SOLVER to fit the data by changing the V_{max} and K_M estimates to minimize the sum of squared residuals.

6.1 If Solver has not been used before, it may not have been installed as described below. If it has been used before skip to step 6.2.

Note: For some users of Excel, add-ins are disabled, therefore Google Sheets has to be used.

FOR EXCEL USERS:

If Solver has not been installed, it can be added to Excel by going to File -> Options -> Add-ins -> Manage Excel Add-ins -> Solver Add-in

FOR GOOGLE SHEETS USERS:

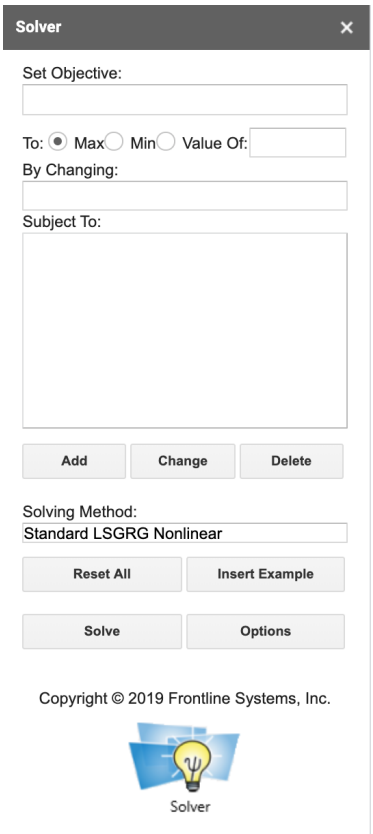
If Solver has not been installed, it can be added to Sheets by going to Add-ons -> Get Add-ons, and searching for Solver.

6.2 Load the Solver window. In Excel, Solver is found in the Data tab. In Sheets, it is found in the Add-ons menu.

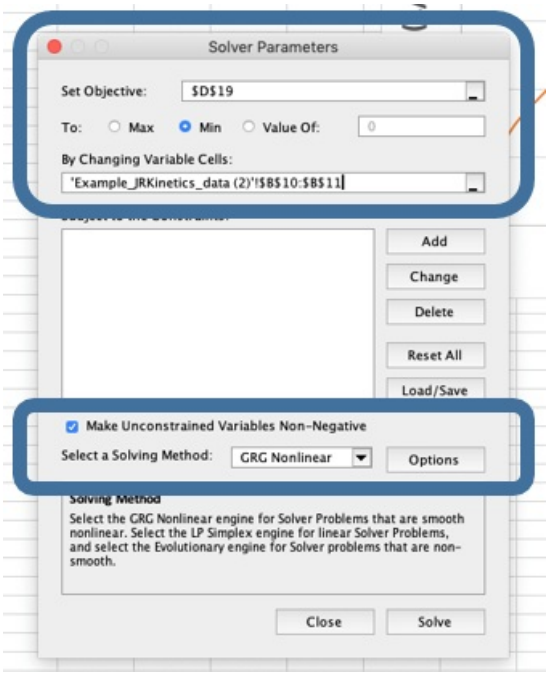
6.3 The Solver window (example from Excel shown below) has 4 parameters to set:

- **Set Objective**: G4
- **To**: Min
- **By Changing Variable Cells**: G2, G3
- **Select a Solving Method**: GRG Nonlinear

The Objective and Variable cells can also be selected using the button at the right of the window and using the cursor to click on the appropriate cells.



Solver in Google Sheets



Solver in Excel

6.4 Click Solve and wait for the fitting to finish (<10 seconds).

Solver will subsitute in various values for V_{max} and K_M to reduce the SSR value as much as possible which means that the theoretical values match the experimental values closely. At the end, the values will be changed automatically in the spreadsheet

6.5 **In Sheets:** Solver will run and change the values.

In Excel: A window will pop-up asking to accept the values and display if there were errors. Click OK and the estimated V_{max} and K_M will be changed to the optimized values.

6.6 Record the values V_{max}, K_M, and SSR below. These V_{max} and K_M values are accurate compared to your original estimates.

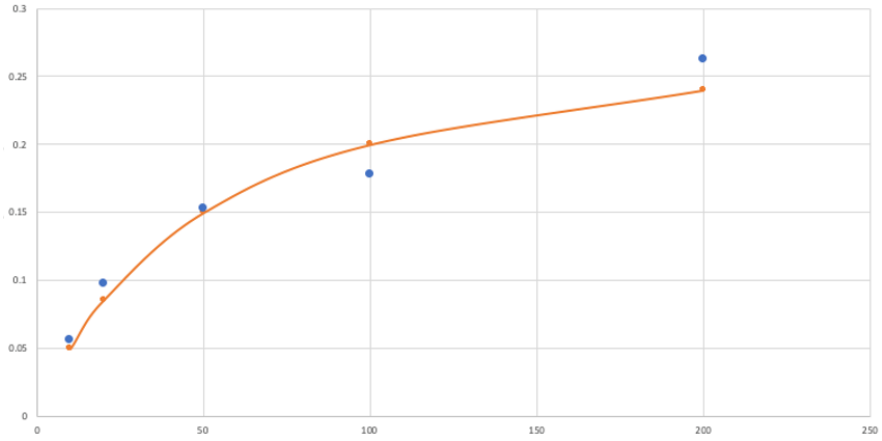
Vmax	
Km	
SSR	1.99566E-07

Visualizing the fit to the data

7 Add a second set of data to the original plot of observed rate vs. concentration of substrate.

7.1 Plot the theoretical (V Calc) rates vs. concentration of substrate.

7.2 Click on the newly plotted V Calc data and edit the data set to show only the line. The plot should look similar to that shown below:



7.3 Label the plot axes with appropriate labels and units.

7.4 Save your plot as a picture and upload the image.