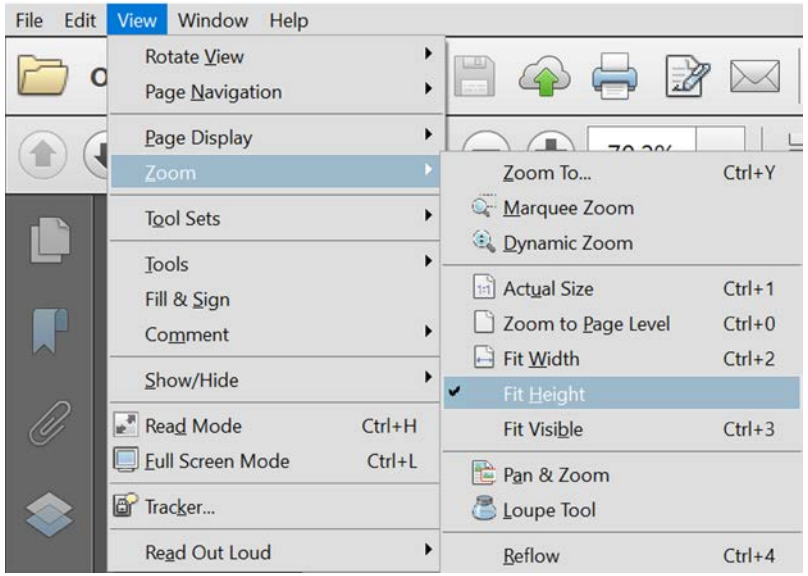
Prism 8 curve fitting to rise-and-fall to steady-state exponential equation

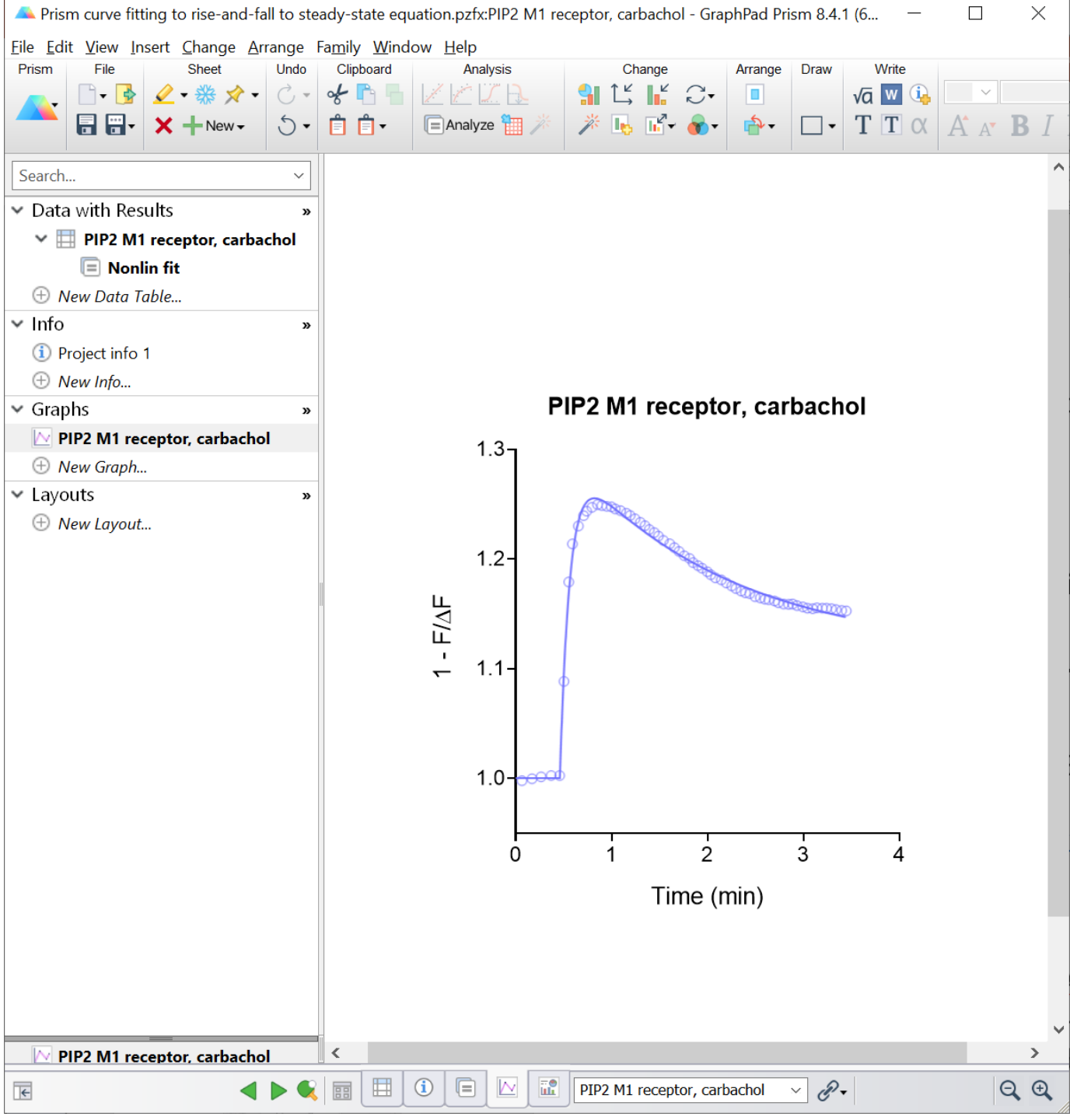


“Fit height” enables page flipping in PDF viewer

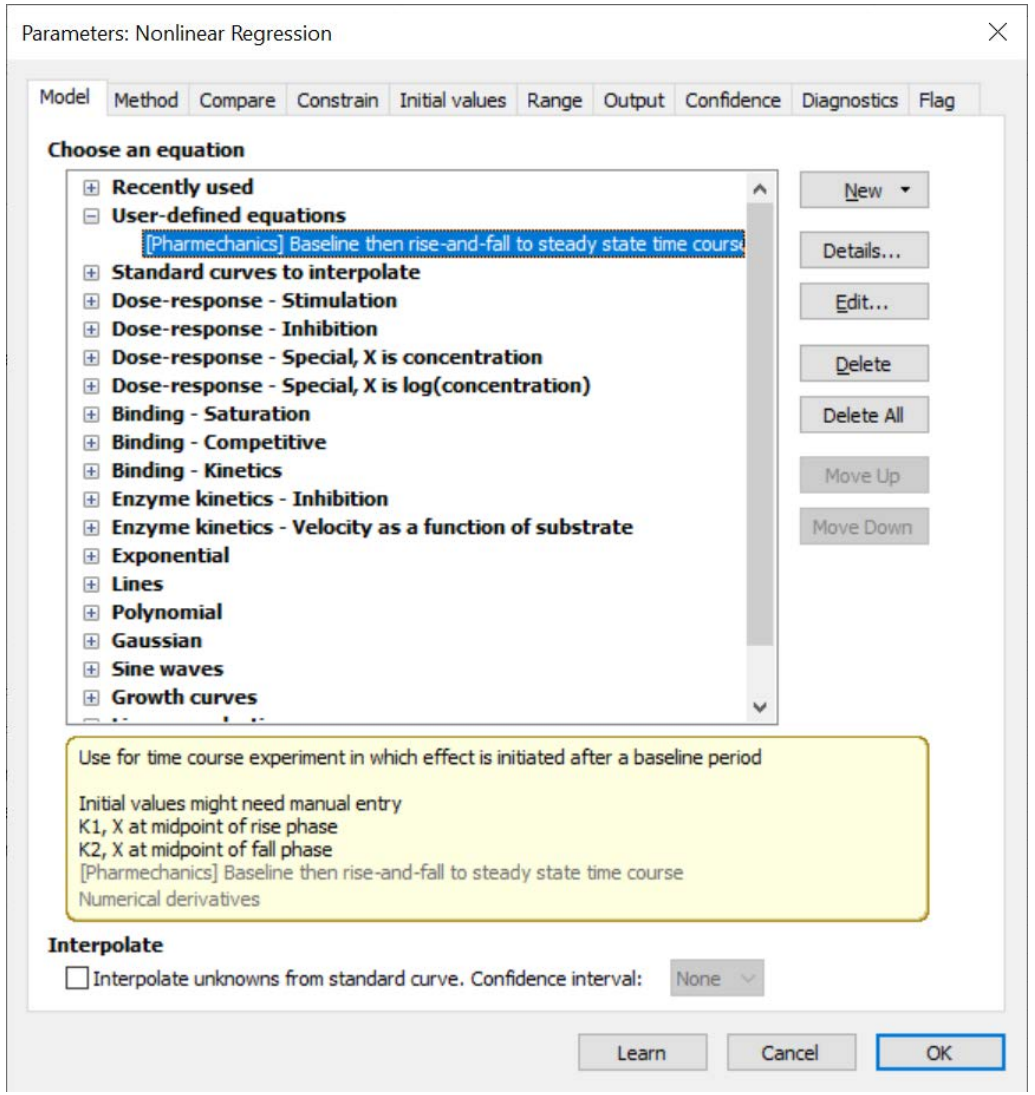
Here we are going to fit biosensor time course data to the rise-and-fall to steady-state equation.

The curve-fitting program is Prism 8, from GraphPad Software, Inc.
https://www.graphpad.com/scientific-software/prism/

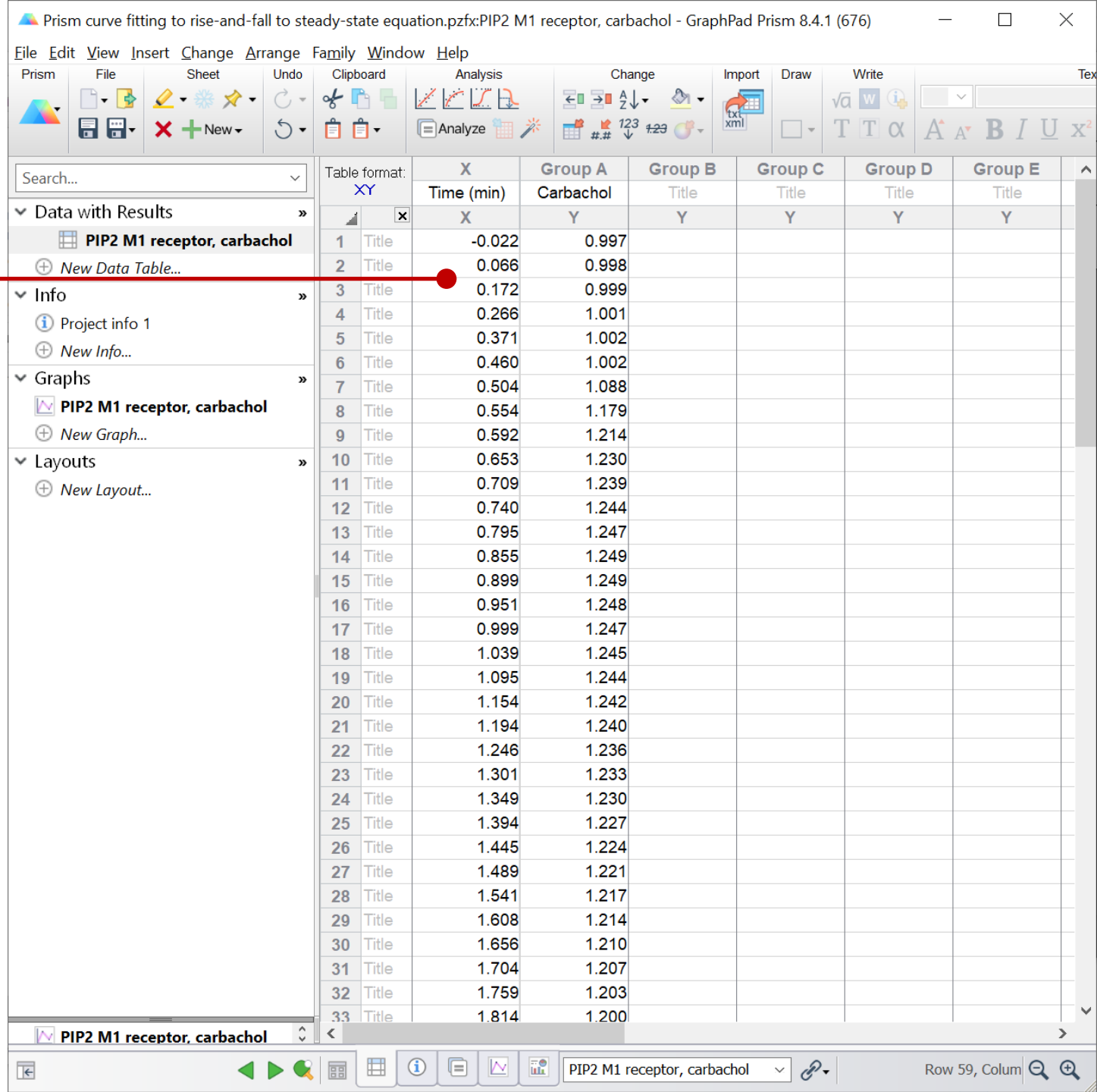
The example will use is PIP2 inhibited by the M1 muscarinic receptor A maximally-stimulating concentration of ligand (carbachol) was used (32 μM).



- First we need to load the equation.
- The rise-and-fall equation with drift is not a built-in equation in Prism.
- Instead it is loaded as a User-defined equation.
- This can be done easily from a template.
- This is shown starting on page 21.
- The equation is called: “[Pharmechanics] Baseline then rise-and-fall to steady-state time course”



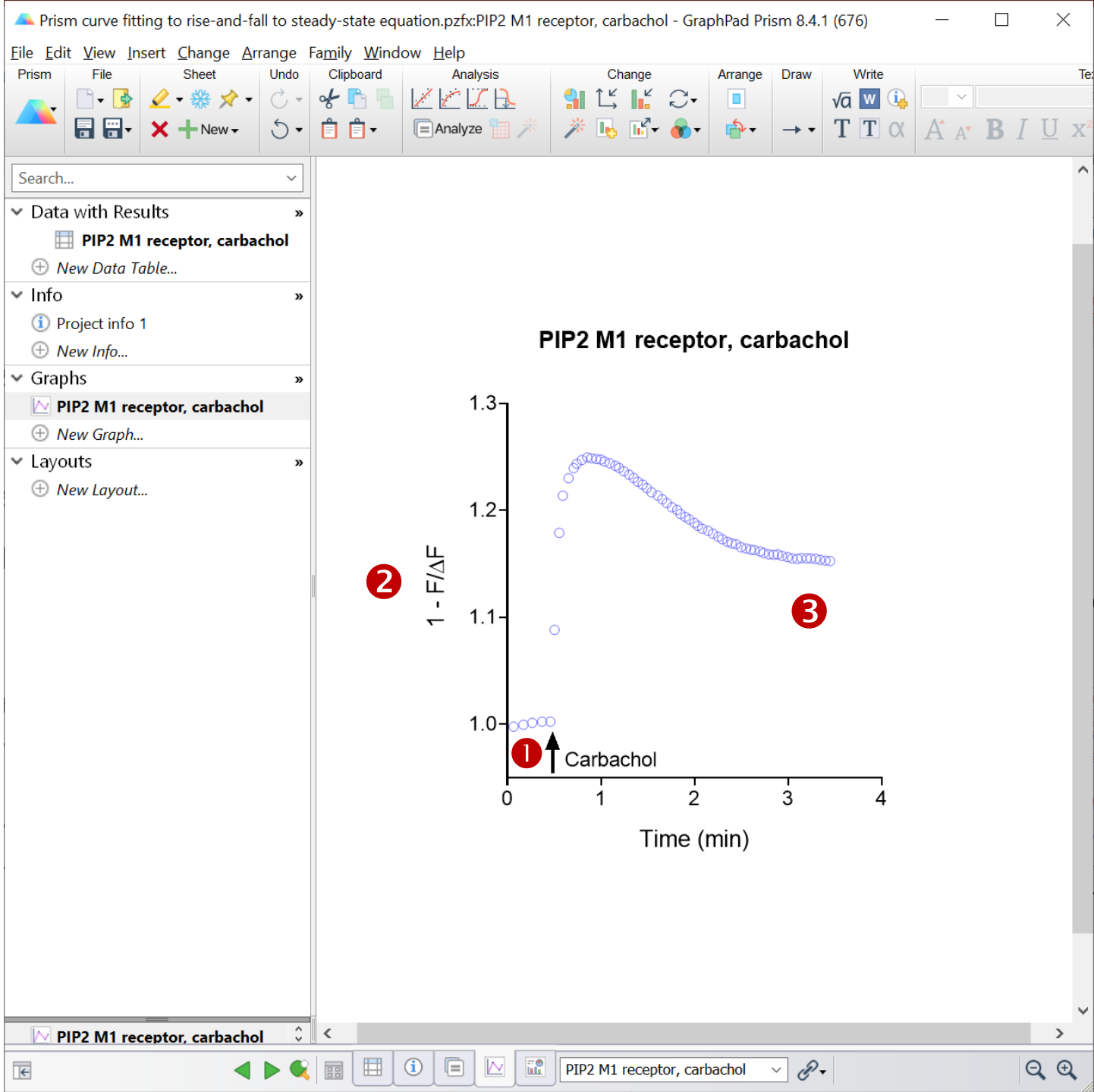
Enter data into Prism data table



Here is the graph of the data.

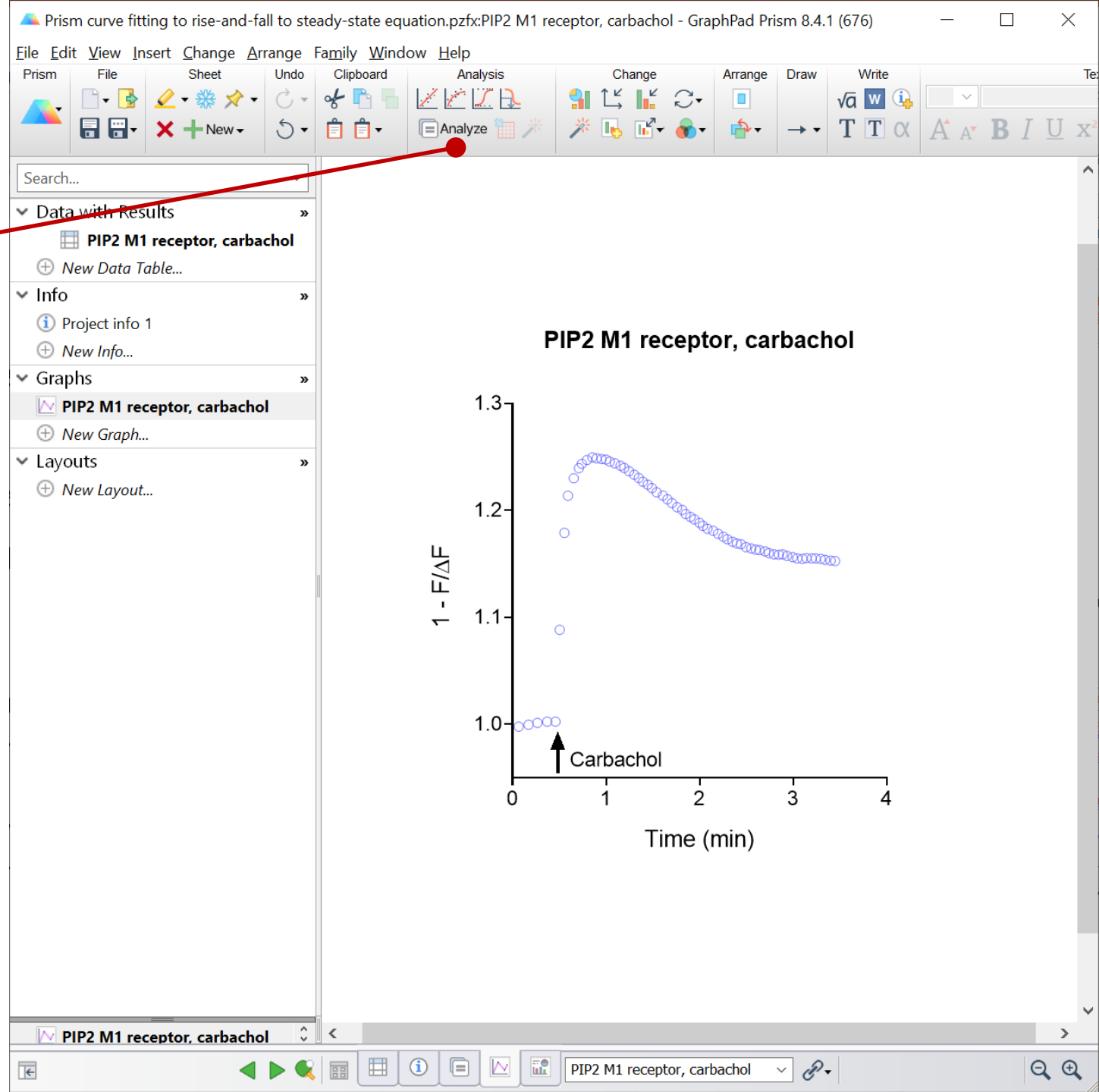
Notes

1. Note lead-in phase before addition of carbachol, where we are measuring the baseline signal.
2. Data are expressed as $\Delta F / F$. This is the fluorescence intensity after carbachol addition divided by the average baseline fluorescence intensity before addition, calculated using a spreadsheet.
3. Note curve plateaus at a steady-state above baseline.



Now we will analyze the data.

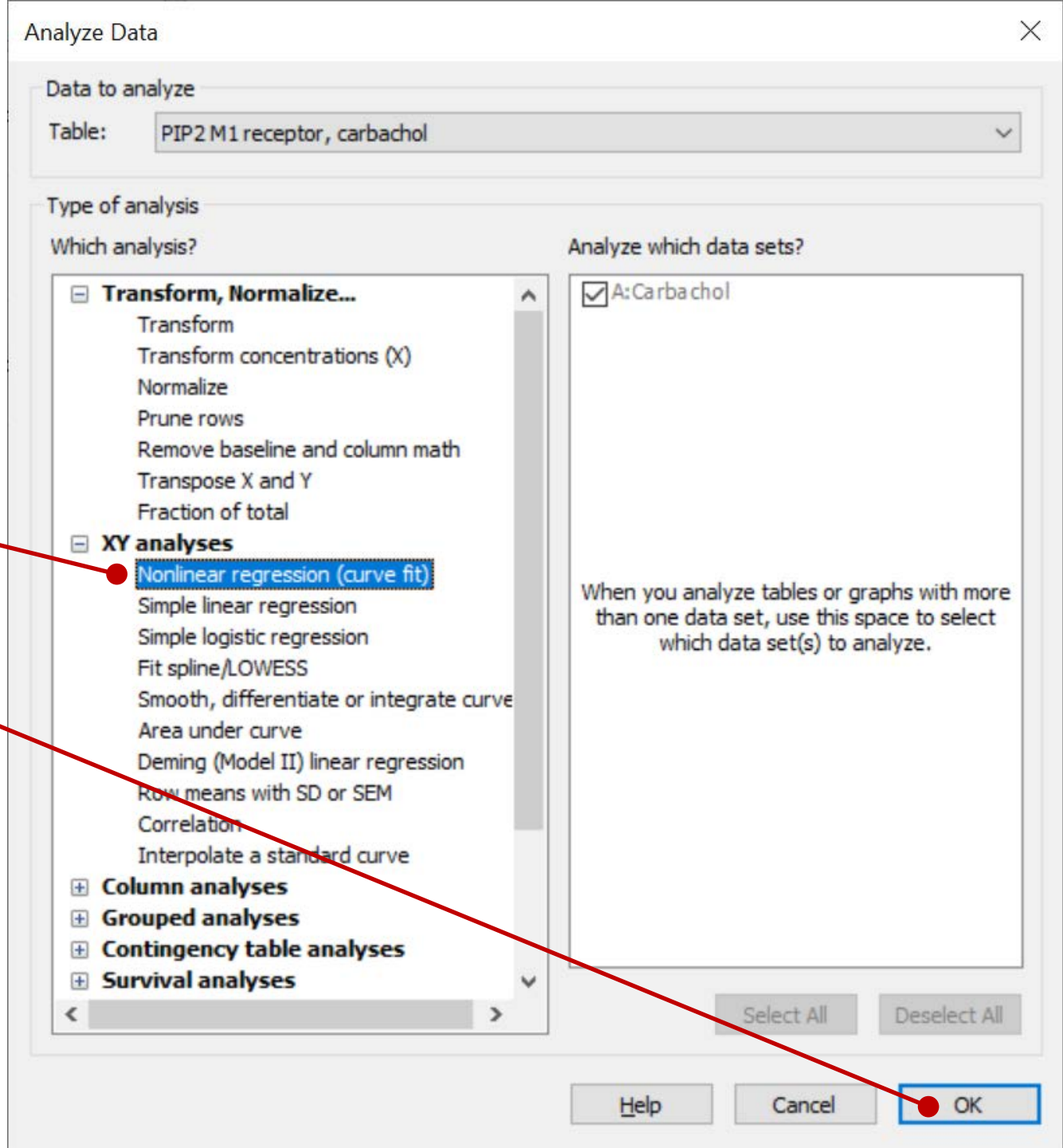
Click “Analyze”



This brings up the “Analyze Data” dialogue

Select “Nonlinear regression”

Click “OK”

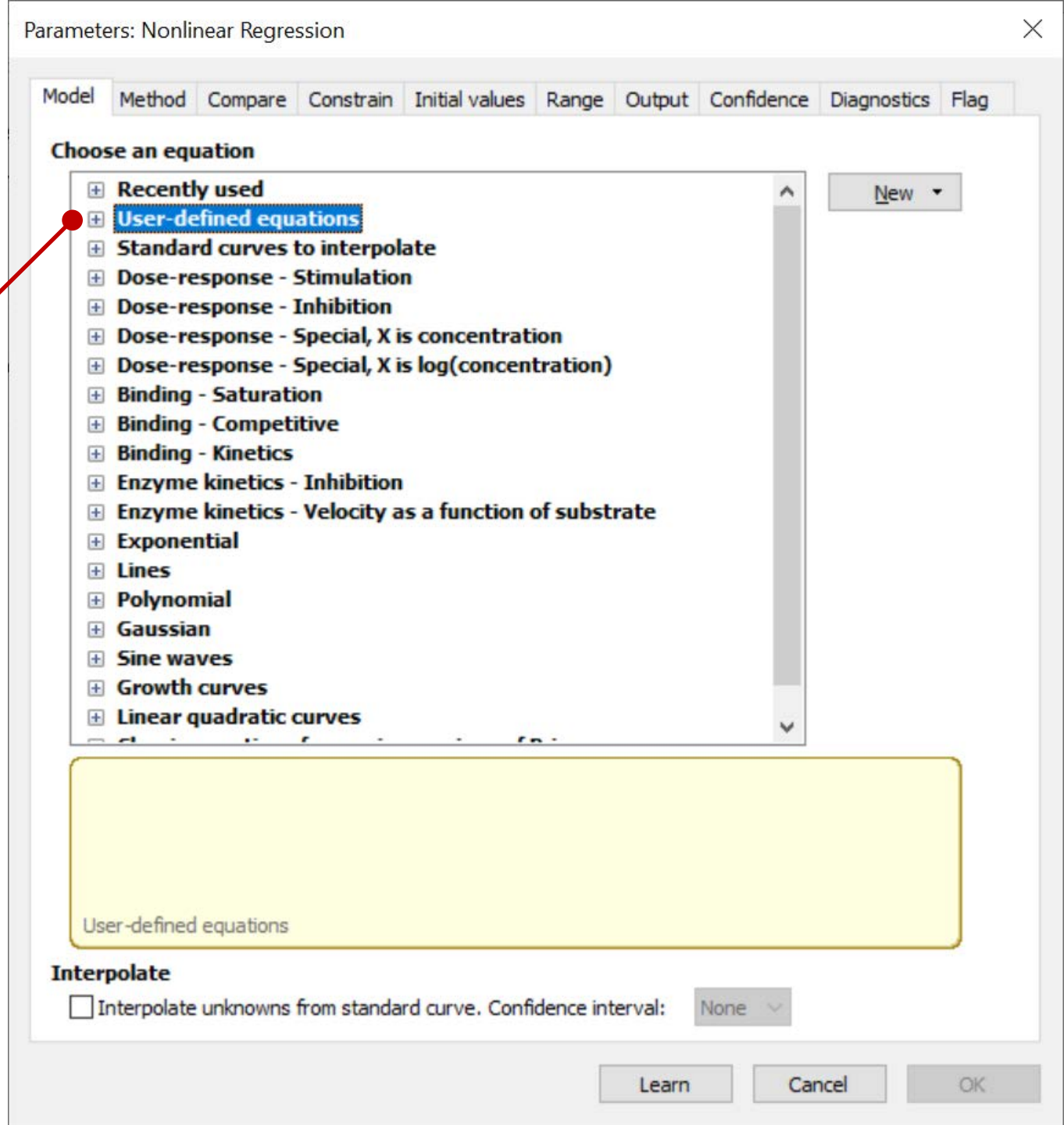


This brings up the “Parameters: Nonlinear Regression” dialogue.

Now we select the equation.

Click “User-defined equations” checkbox.

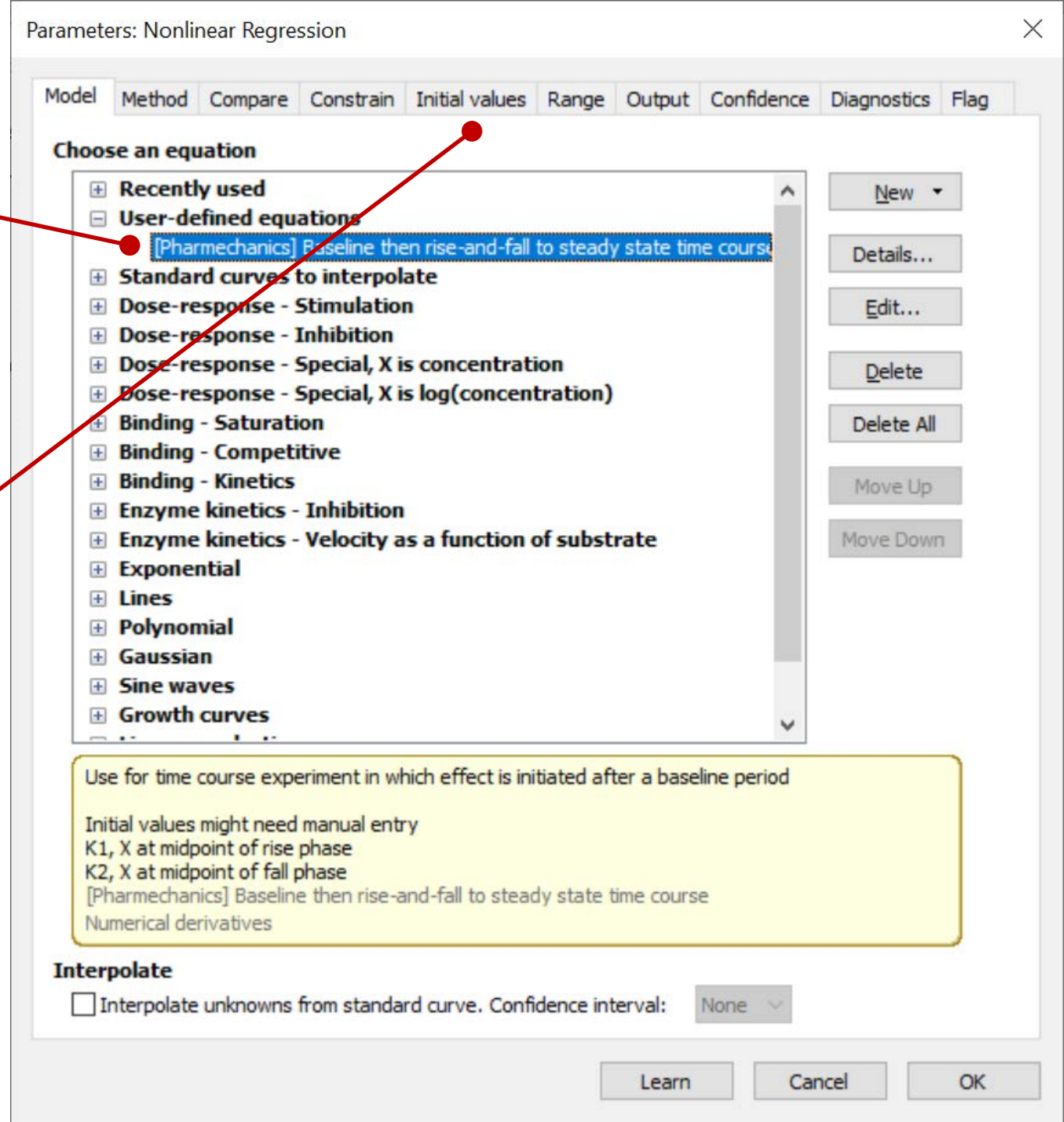
Note this will only appear if user-defined equations have been loaded. See page 21.



Select ““[Pharmechanics] Baseline then rise-and-fall to steady-state time course”

Note this will only appear if the equation has been loaded. See page 21.

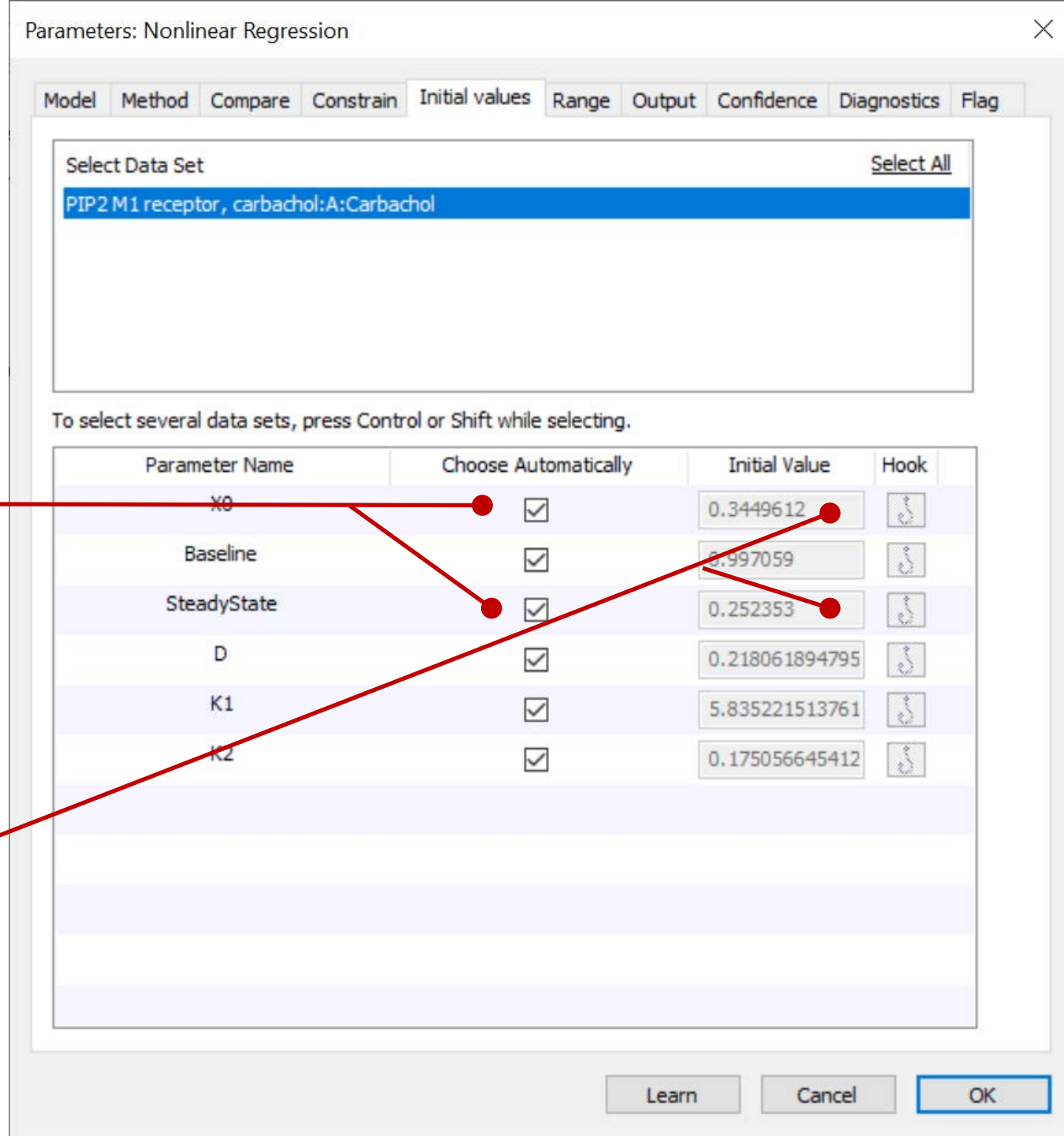
Click on the “Initial values” tab



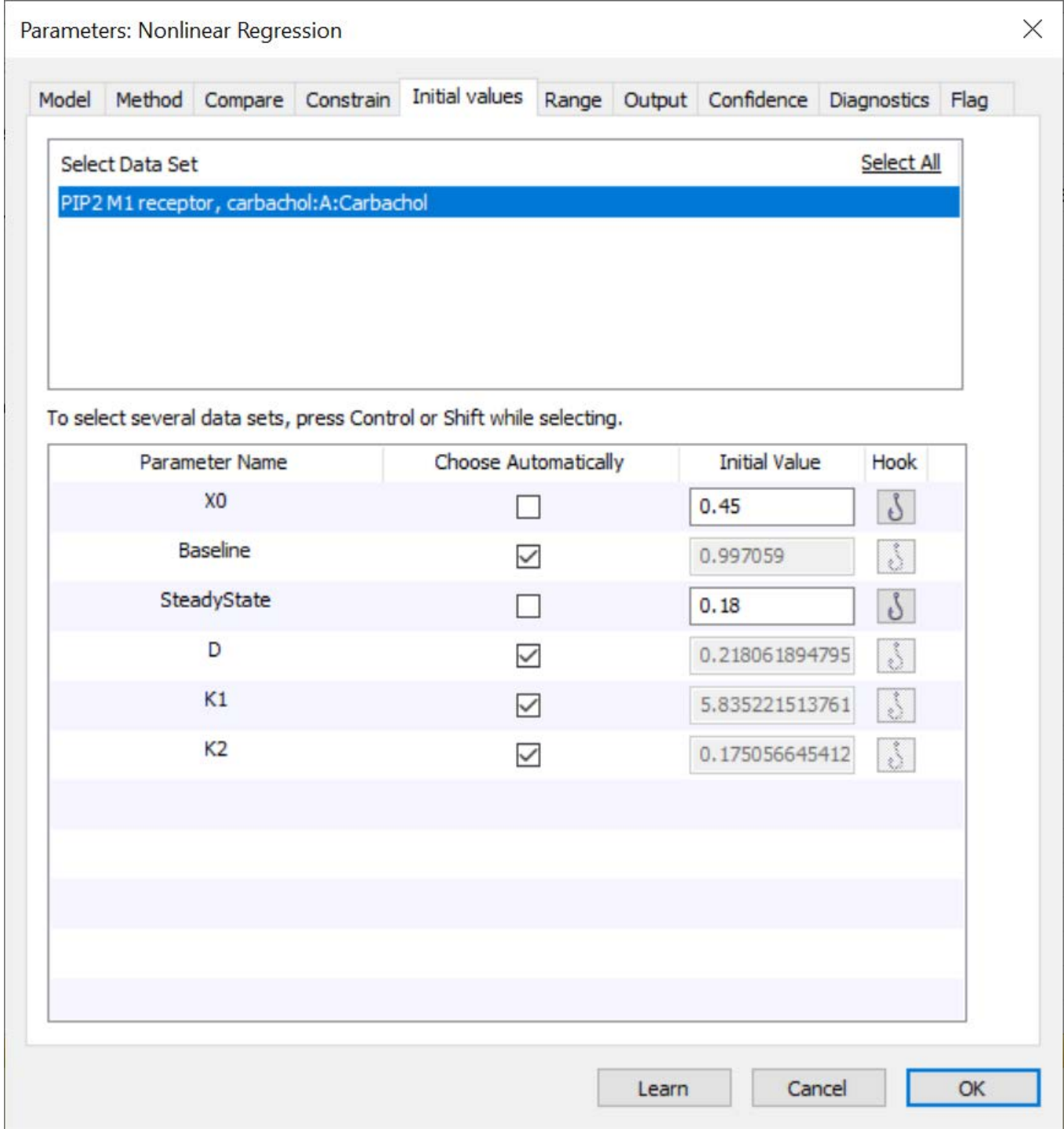
Here we recommend manually entering X0 (the time the signal starts to rise) and SteadyState (the asymptote minus baseline).

Uncheck “Choose Automatically” for X0 and SteadyState.

Enter the estimated X0 and SteadyState values (from visual inspection of the graph)



These are the values for this experiment.

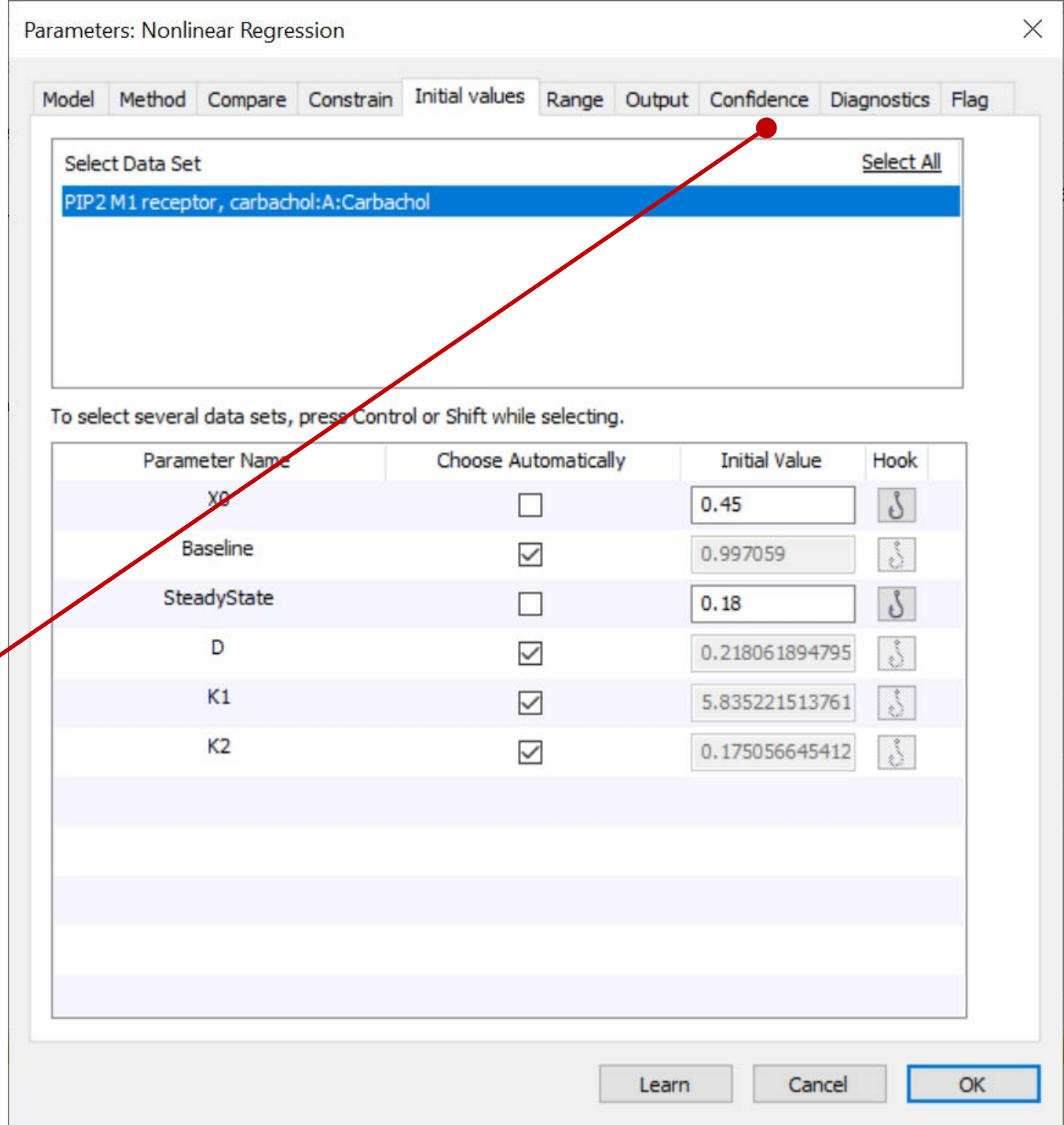


Now we are going to change a setting to make the analysis run efficiently.

Prism uses a rigorous method to compute the error associated with the fitted parameter values. For complicated equations this can greatly increase the fitting time.

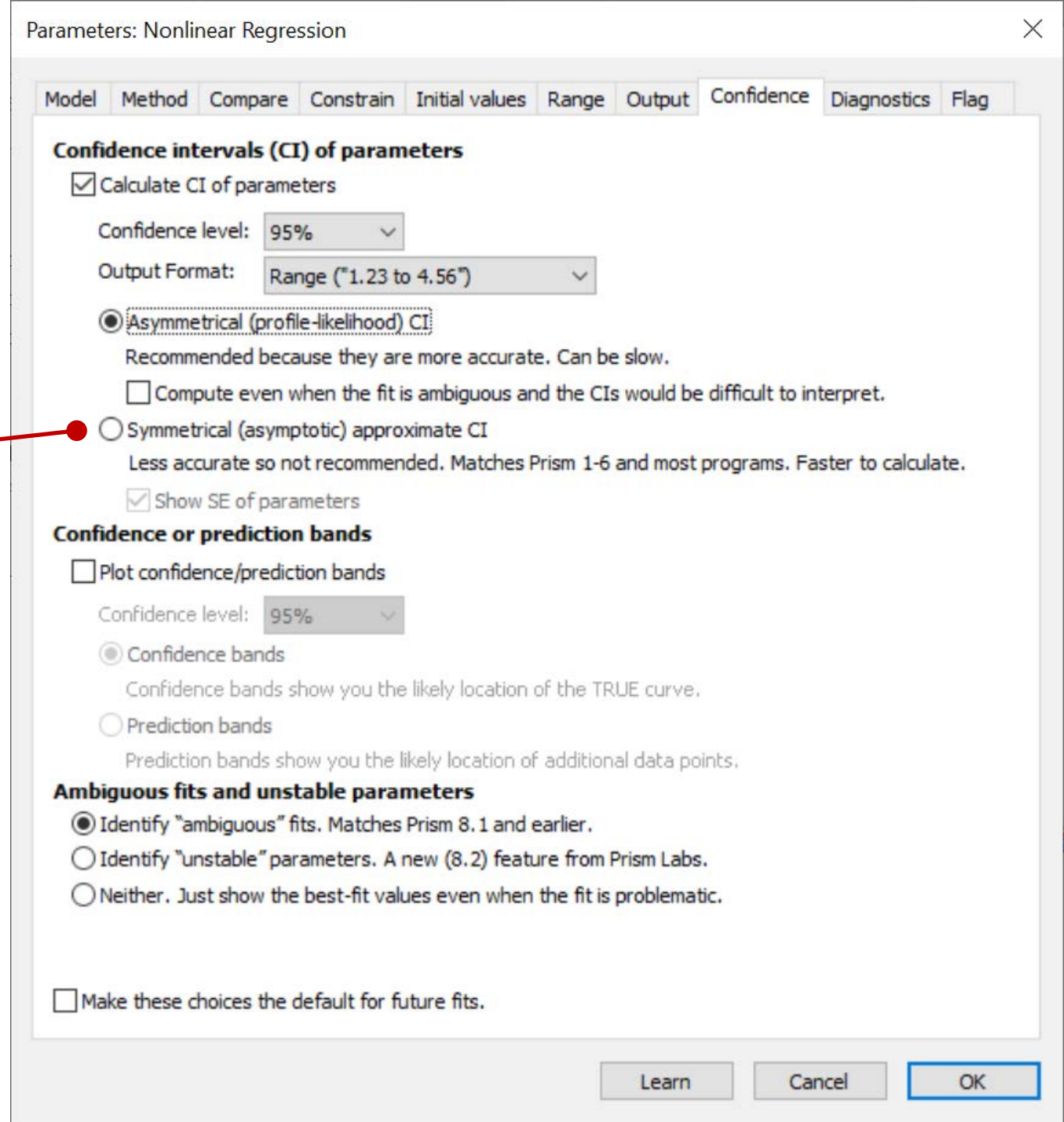
The rigorous method can be turned off to make the analysis run faster.

Click on the “Confidence” tab

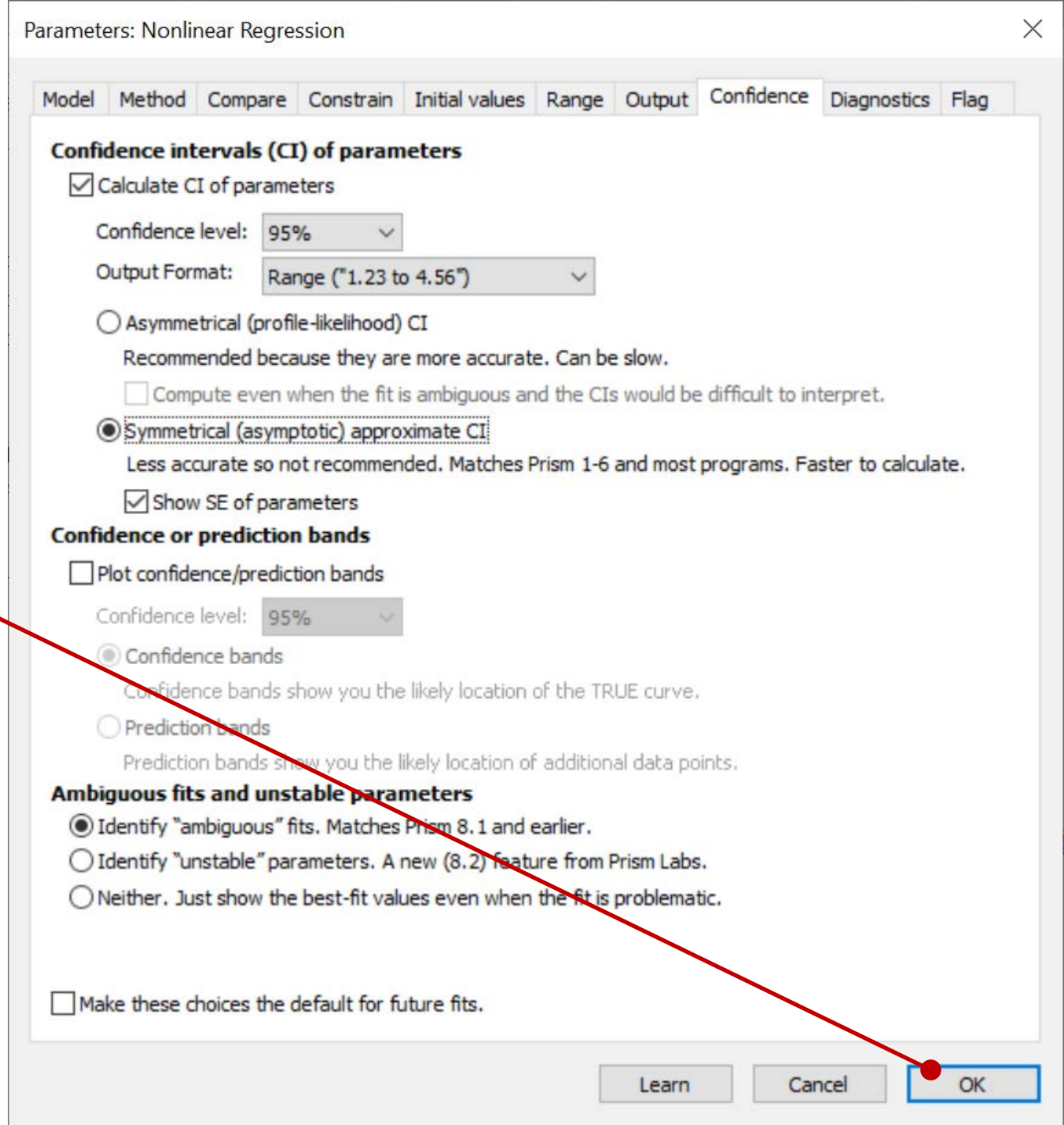


"Asymmetrical" is the rigorous error calculation method

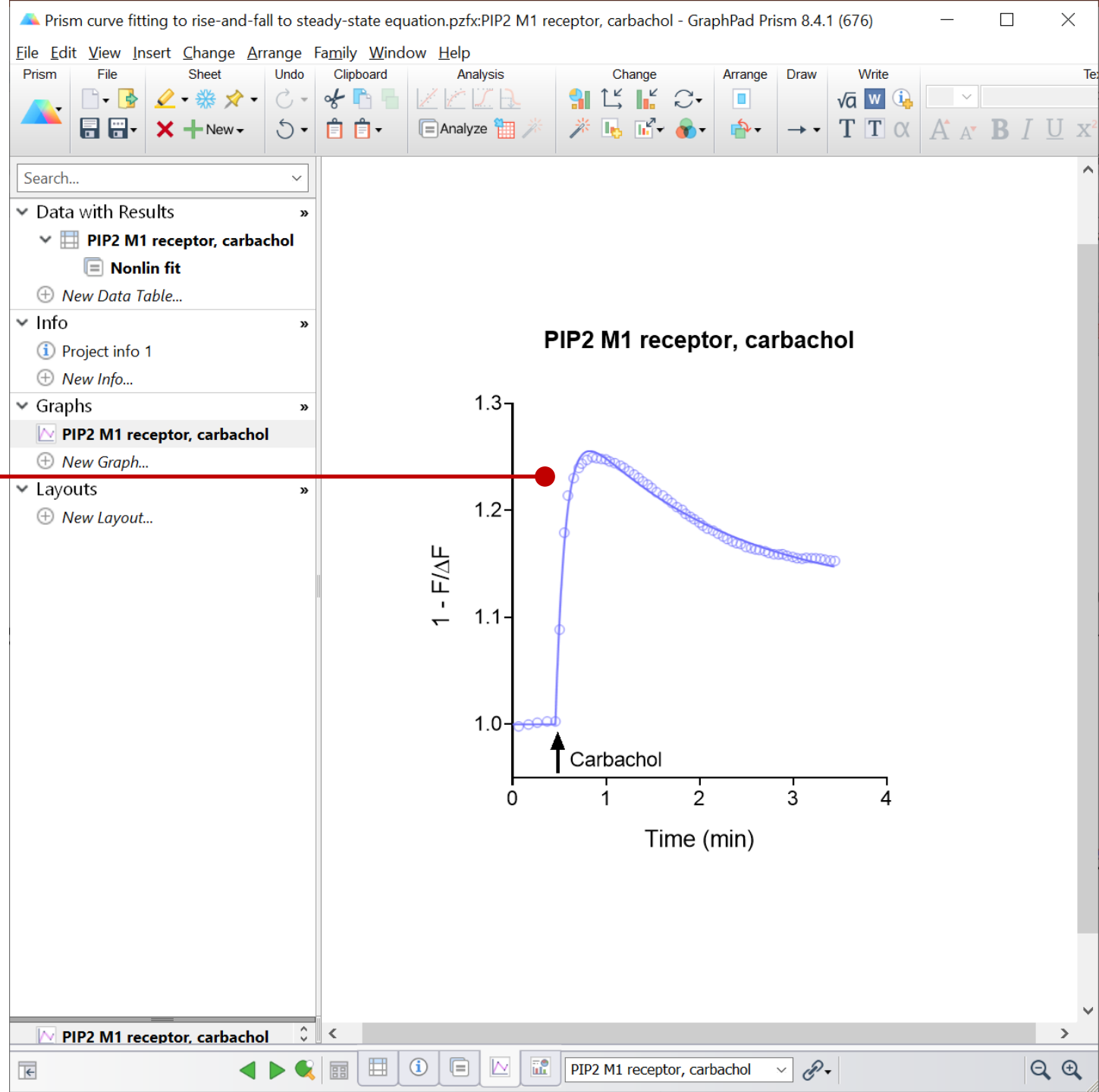
To turn it off, click the "Symmetrical" radio button



Click “OK” to run the analysis

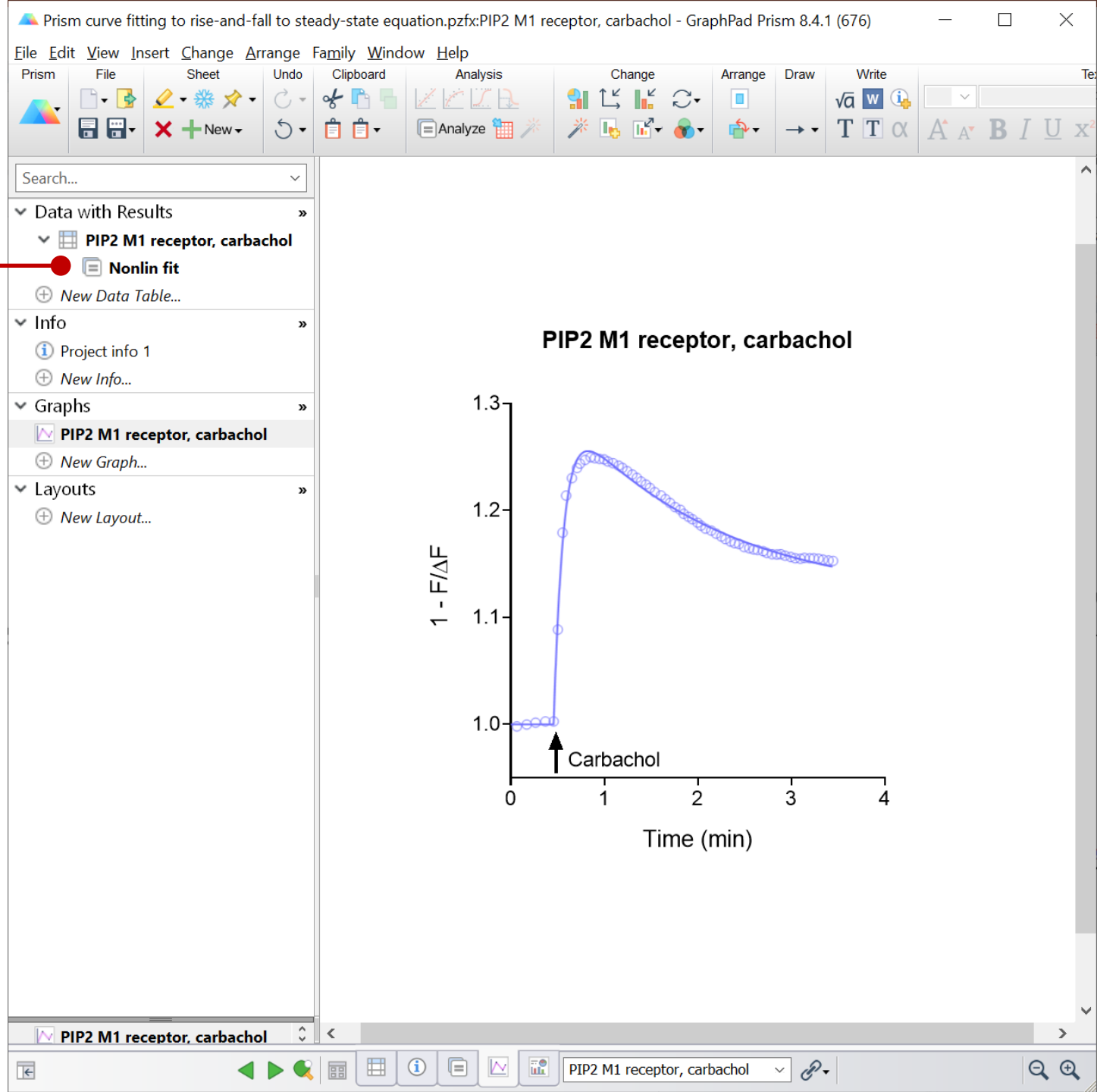


This is the fitted curve



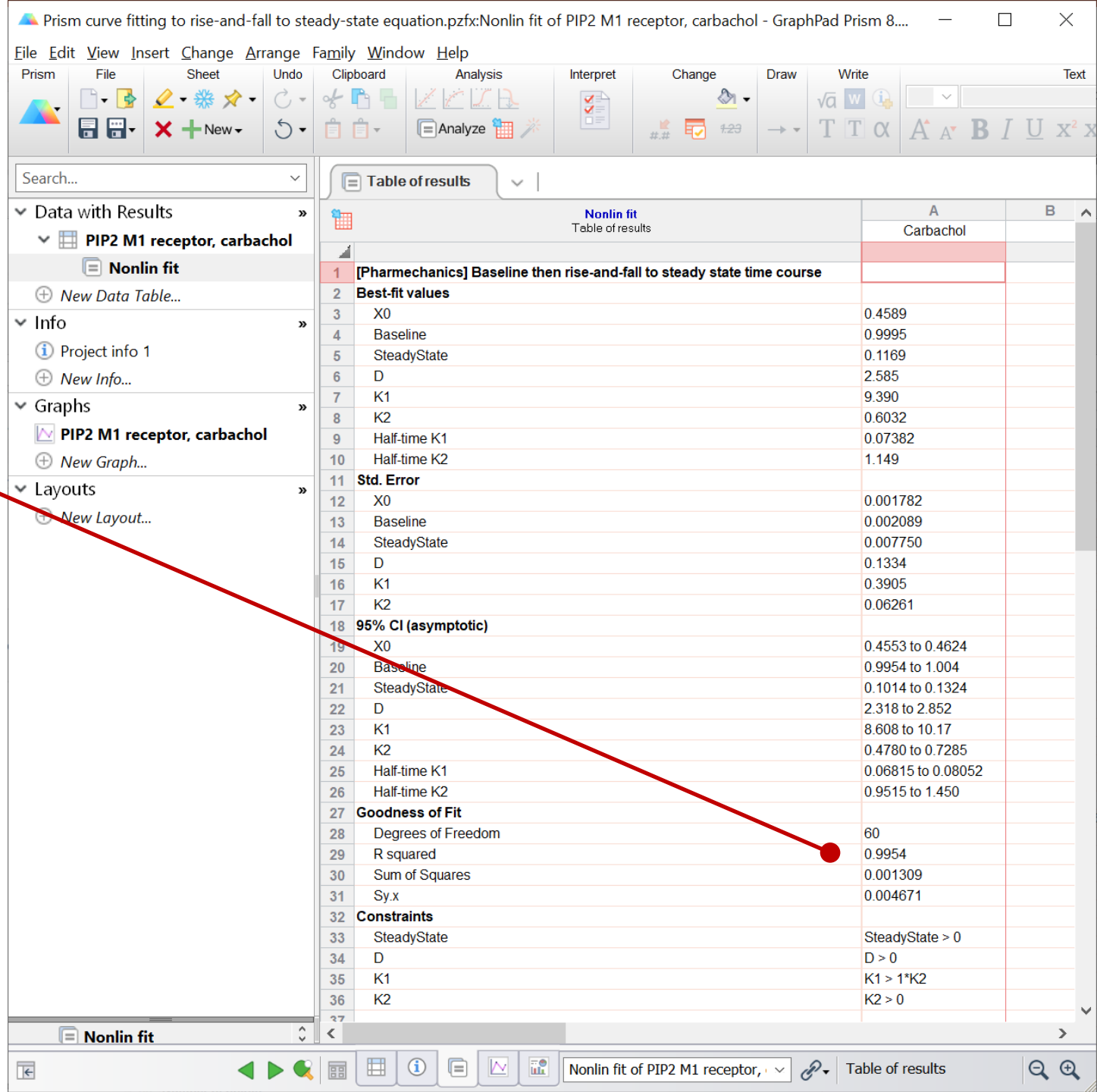
Reviewing the results

Click “Nonlin fit” to bring up the results table



Look at the R squared value to determine the goodness of fit

Data are fit well by the equation: $R^2 = 0.995$



Calculating the initial rate and k_τ

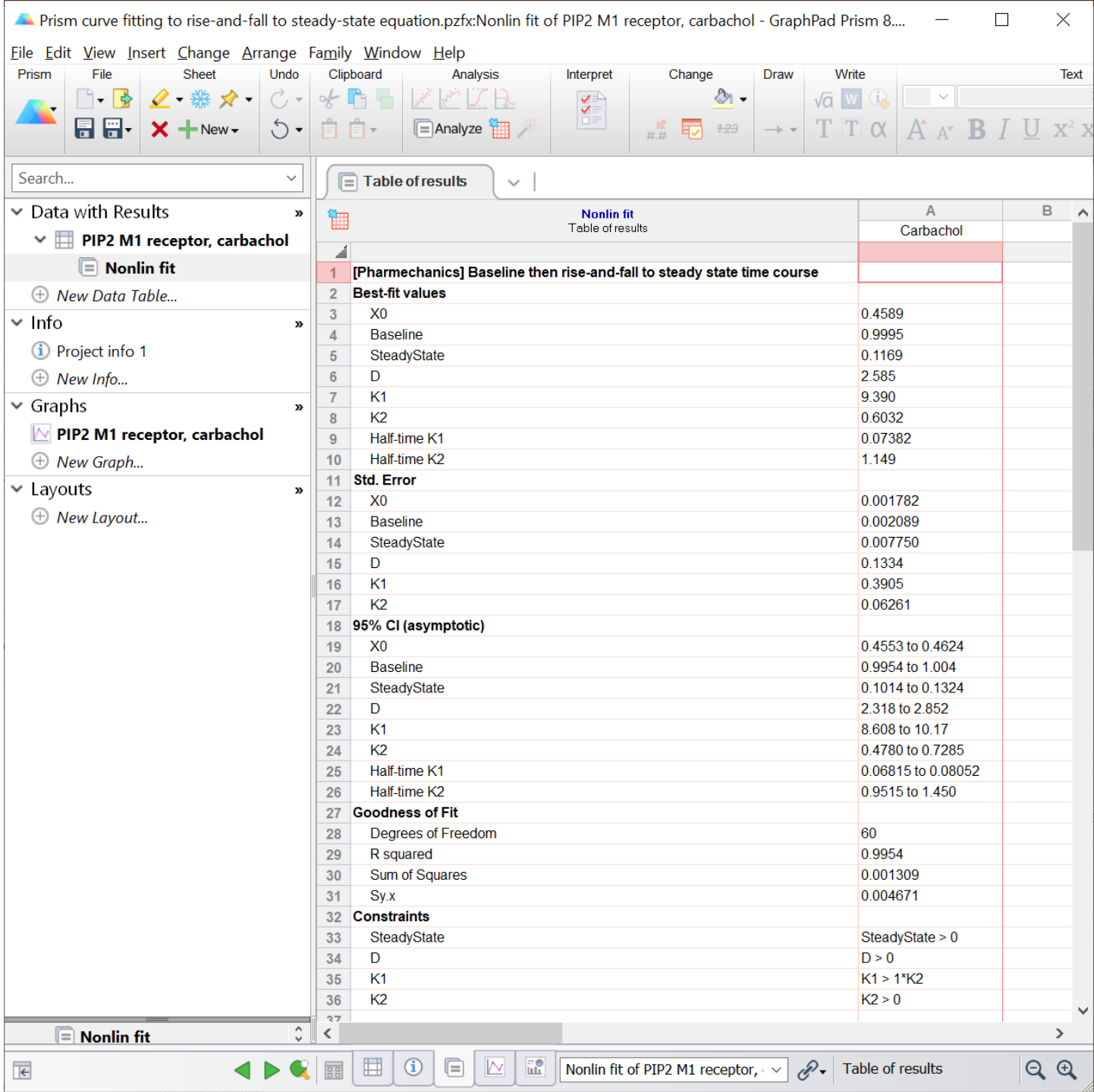
Use this formula to calculate the initial rate:

$$\text{Initial rate} = \text{SSR} \times (Dk_1 - (D-1)k_2)$$

Initial rate = 2.73 NFU.min^{-1}

Since we are using a maximally-stimulating concentration of ligand, k_τ is equal to the initial rate,

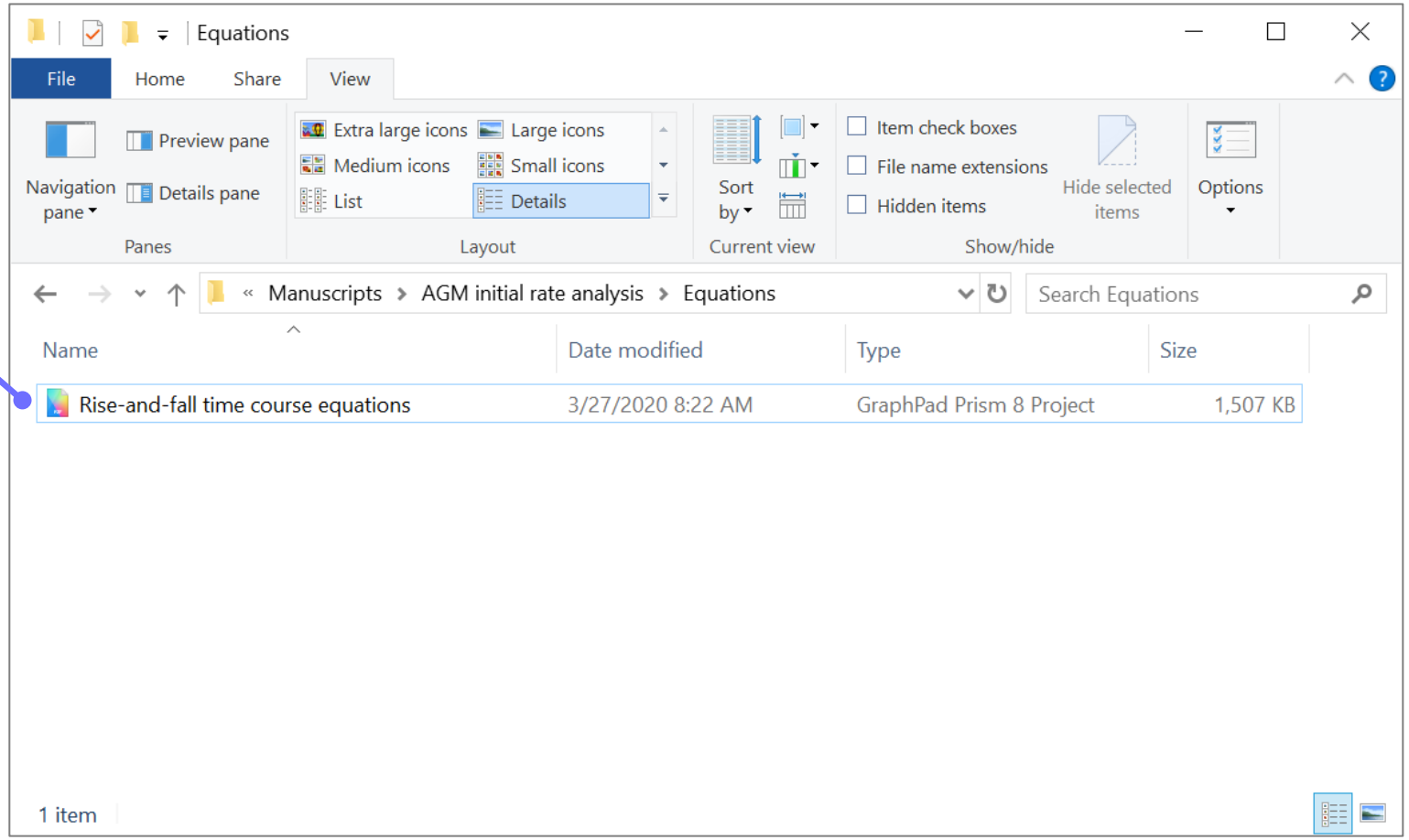
NFU, normalized fluorescence units



Loading equations into Prism from a file

First, download the file containing the equations to your computer.

Then open it.

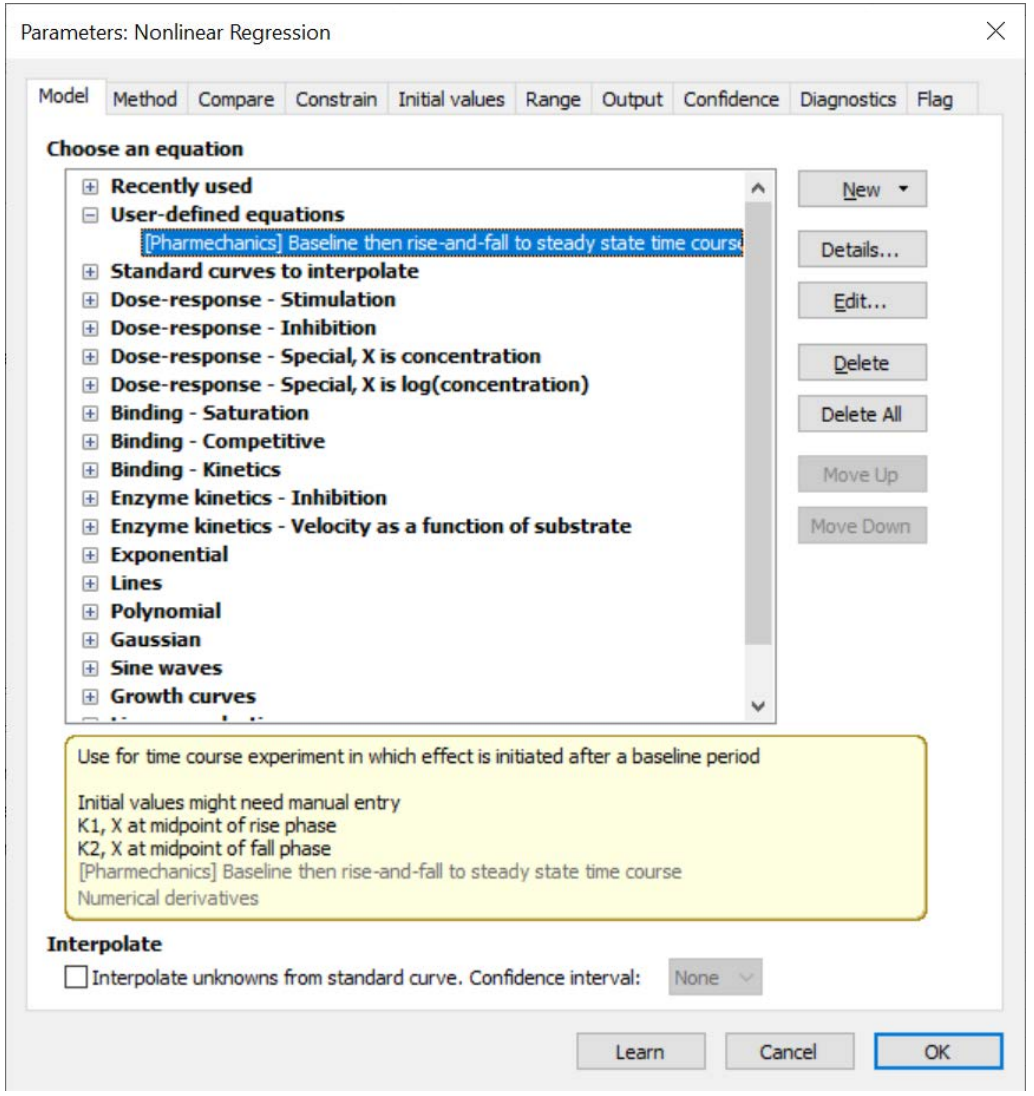


GraphPad Prism contains an equation editor for the input of user-defined equations.

There is a sharing method that simplifies the loading of equations written by other users.

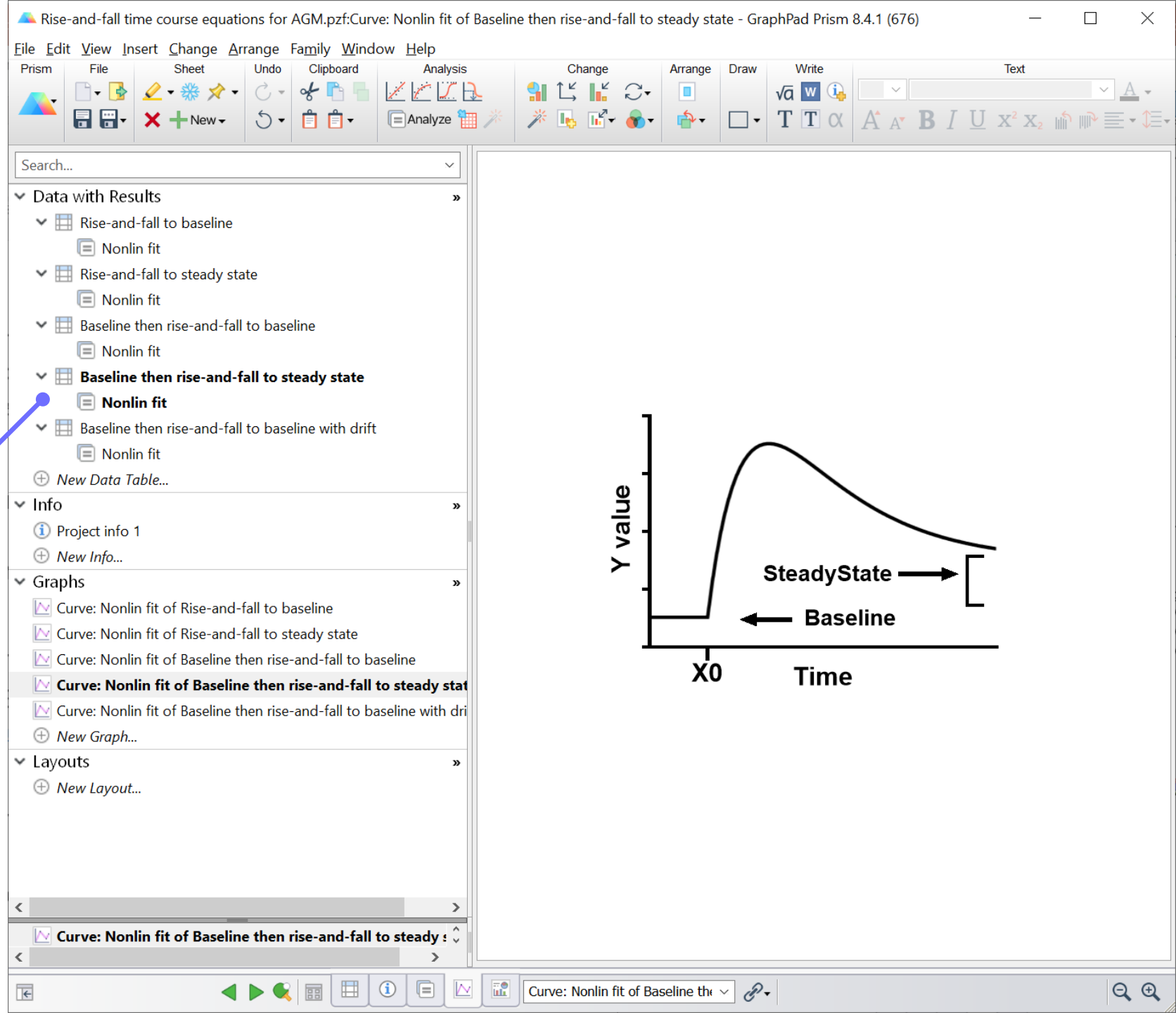
This avoids the need to write in the equation and all the fit settings.

This process is described in this presentation.

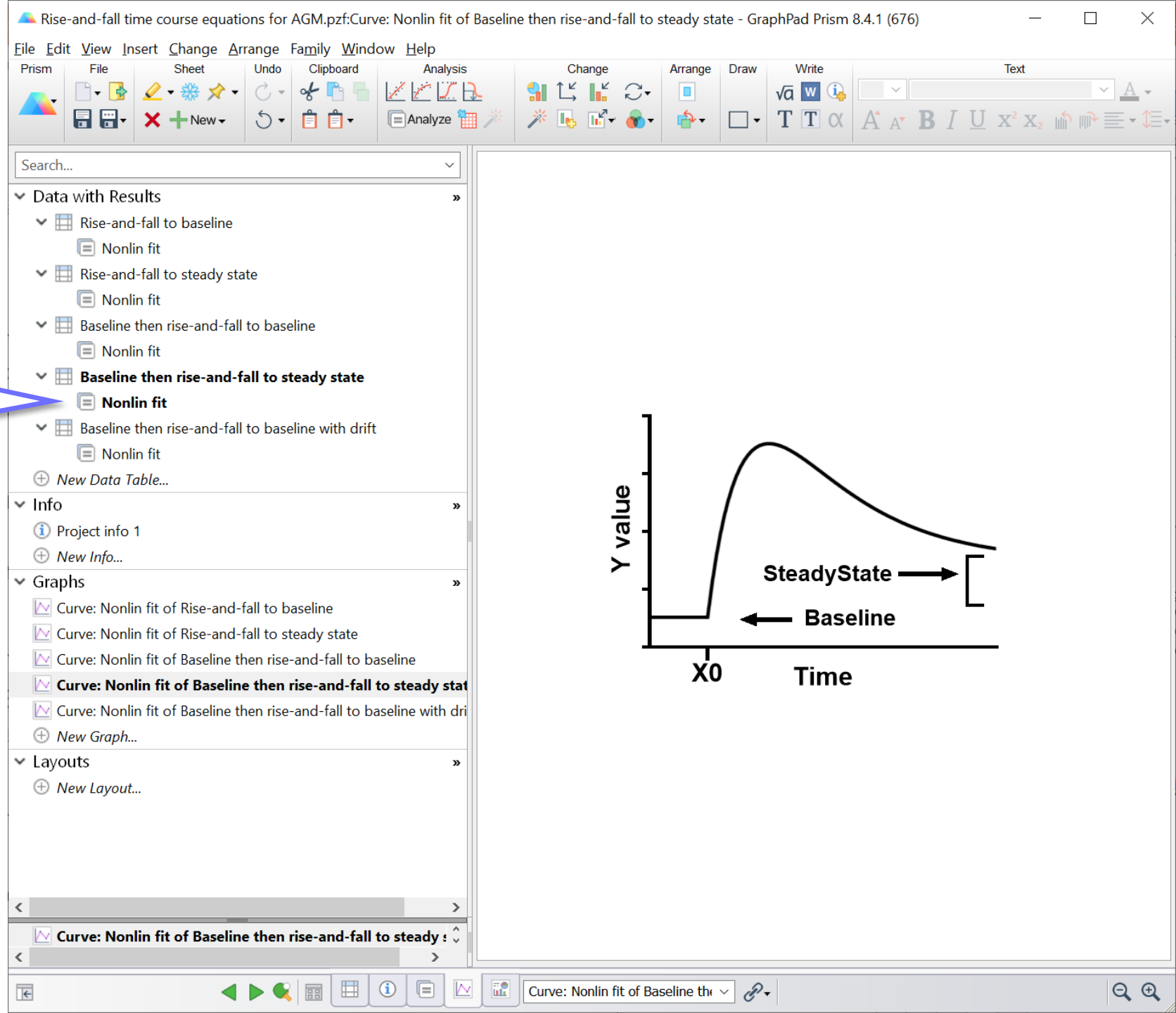


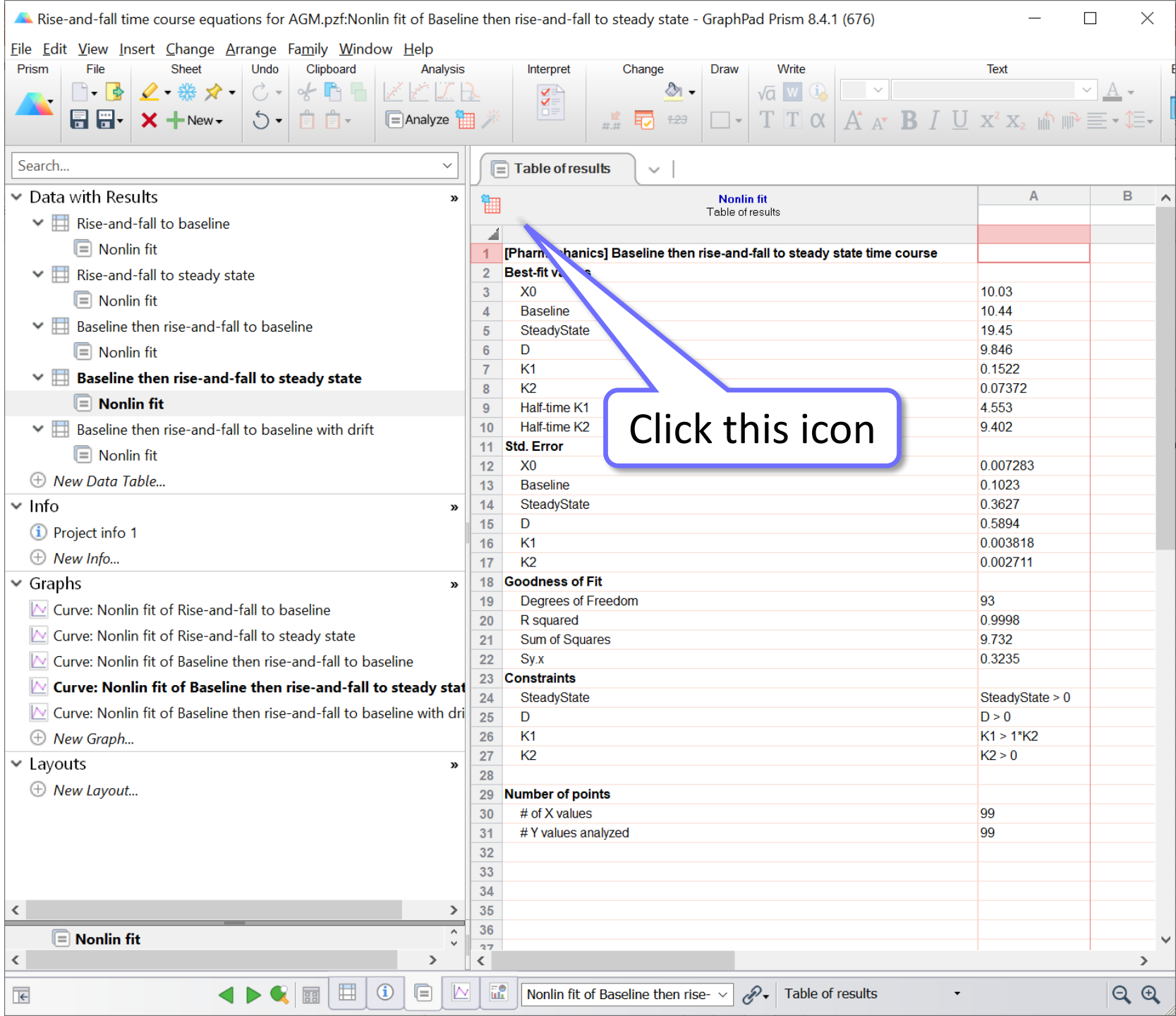
We are going to load the equation from the Results sheet.

The equation we need is called “Baseline then rise-and-fall to steady-state”



Select the equation you want by clicking on the “Nonlin fit” Results tab.




Rise-and-fall time course equations for AGM.pzf:Nonlin fit of Baseline then rise-and-fall to steady state - GraphPad Prism 8.4.1 (676)
File Edit View Insert Change Arrange Family Window Help
Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text
New
Analyze
Search...
Data with Results
Rise-and-fall to baseline
Nonlin fit
Rise-and-fall to steady state
Nonlin fit
Baseline then rise-and-fall to baseline
Nonlin fit
Baseline then rise-and-fall to steady state
Nonlin fit
Baseline then rise-and-fall to baseline with drift
Nonlin fit
New Data Table...
Info
Project info 1
New Info...
Graphs
Curve: Nonlin fit of Rise-and-fall to baseline
Curve: Nonlin fit of Rise-and-fall to steady state
Curve: Nonlin fit of Baseline then rise-and-fall to baseline
Curve: Nonlin fit of Baseline then rise-and-fall to steady stat
Curve: Nonlin fit of Baseline then rise-and-fall to baseline with dri
New Graph...
Layouts
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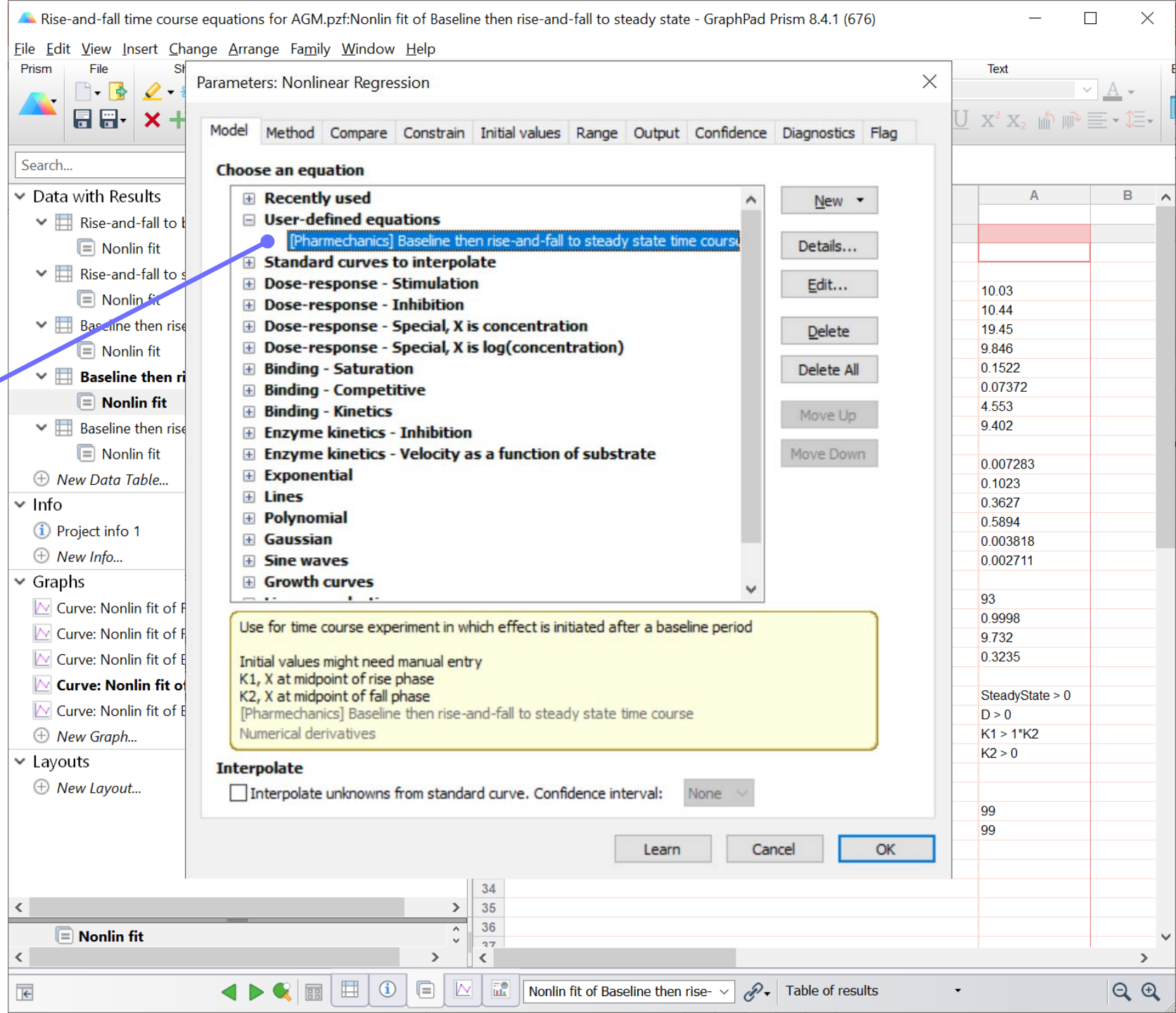
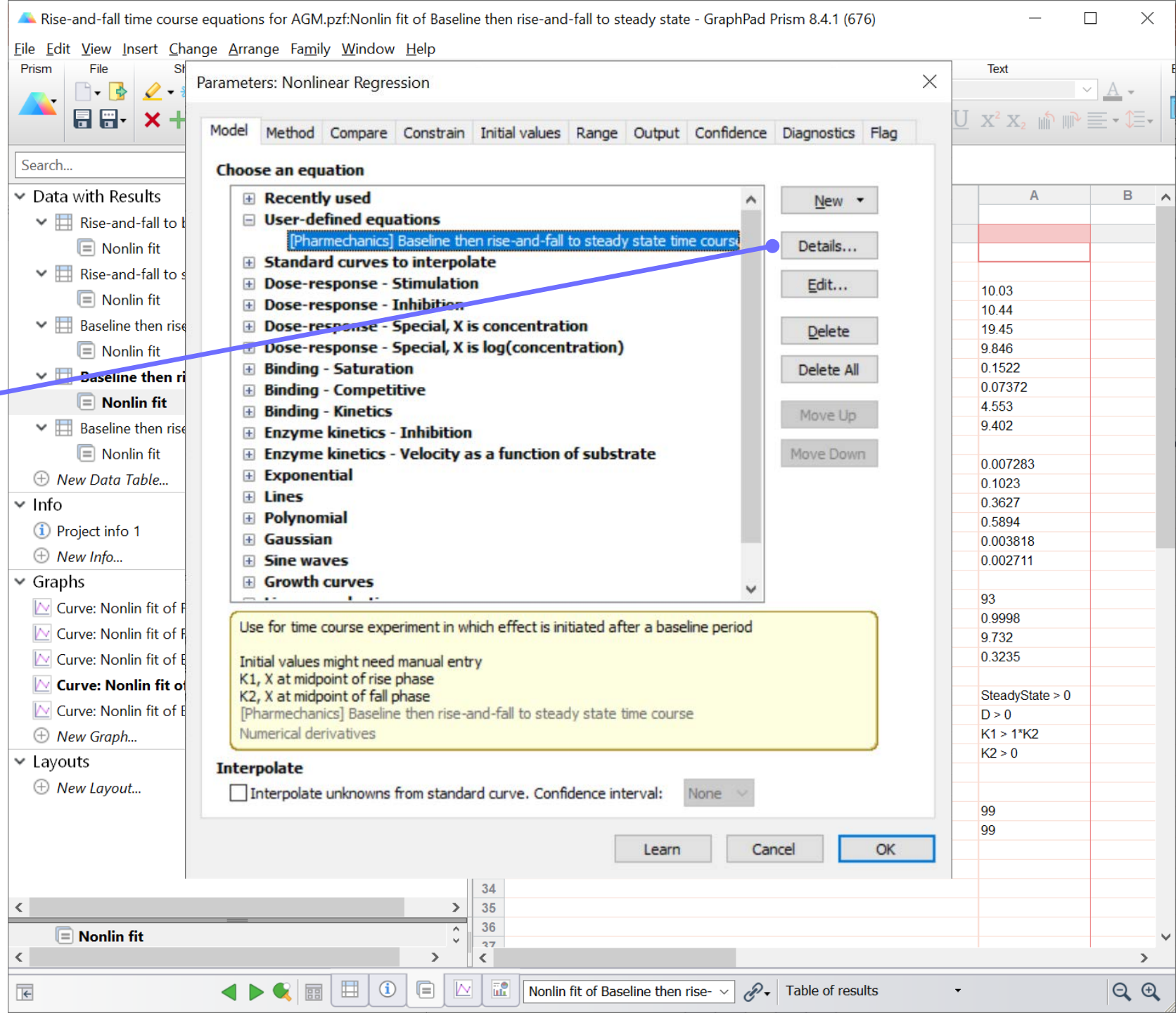
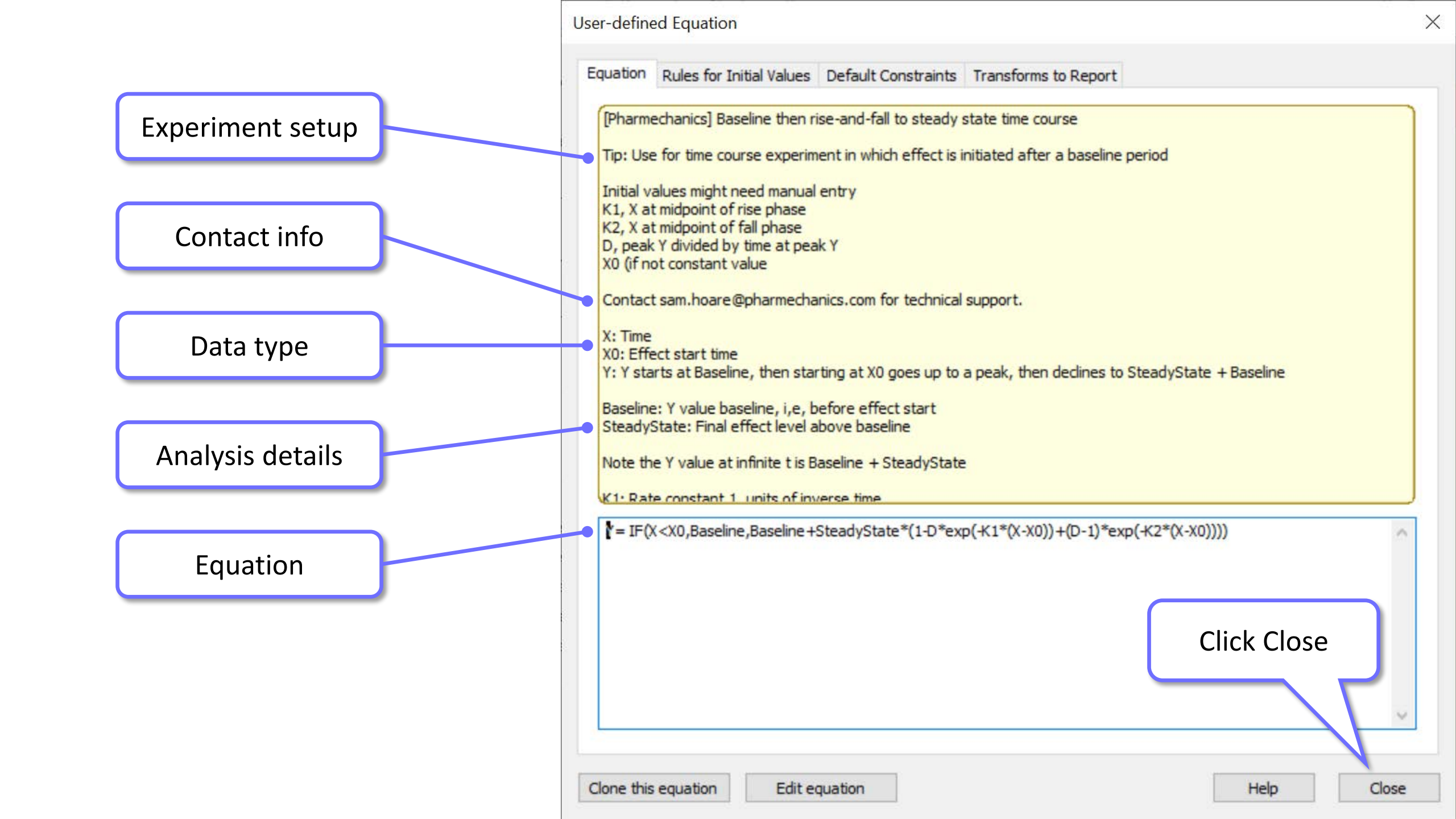
Nonlin fit
Table of results
Nonlin fit
Table of results
A
B
1 [Pharm hanics] Baseline then rise-and-fall to steady state time course
2 Best-fit v s
3 X0 10.03
4 Baseline 10.44
5 SteadyState 19.45
6 D 9.846
7 K1 0.1522
8 K2 0.07372
9 Half-time K1 4.553
10 Half-time K2 9.402
11 Std. Error
12 X0 0.007283
13 Baseline 0.1023
14 SteadyState 0.3627
15 D 0.5894
16 K1 0.003818
17 K2 0.002711
18 Goodness of Fit
19 Degrees of Freedom 93
20 R squared 0.9998
21 Sum of Squares 9.732
22 Sy.x 0.3235
23 Constraints
24 SteadyState SteadyState > 0
25 D D > 0
26 K1 K1 > 1*K2
27 K2 K2 > 0
28
29 Number of points
30 # of X values 99
31 # Y values analyzed 99
32
33
34
35
36
Click this icon
Nonlin fit of Baseline then rise-
Table of results

This process loads the equation into the “User-defined equations” list. It only needs to be done once. After that, the new equation will be available every time you open Prism.



Click “Details” for guidance on how to use the equation.




Experiment setup
Contact info
Data type
Analysis details
Equation
User-defined Equation
Equation
Rules for Initial Values
Default Constraints
Transforms to Report
[Pharmechanics] Baseline then rise-and-fall to steady state time course
Tip: Use for time course experiment in which effect is initiated after a baseline period
Initial values might need manual entry
K1, X at midpoint of rise phase
K2, X at midpoint of fall phase
D, peak Y divided by time at peak Y
X0 (if not constant value
Contact sam.hoare@pharmechanics.com for technical support.
X: Time
X0: Effect start time
Y: Y starts at Baseline, then starting at X0 goes up to a peak, then declines to SteadyState + Baseline
Baseline: Y value baseline, i,e, before effect start
SteadyState: Final effect level above baseline
Note the Y value at infinite t is Baseline + SteadyState
K1: Rate constant 1, units of inverse time
Y = IF(X<X0,Baseline,Baseline+SteadyState*(1-D*exp(-K1*(X-X0))+(D-1)*exp(-K2*(X-X0))))
Click Close
Clone this equation
Edit equation
Help
Close

Click OK then close file. Now the equation is available every time you open Prism, in the “User-defined equations” list.