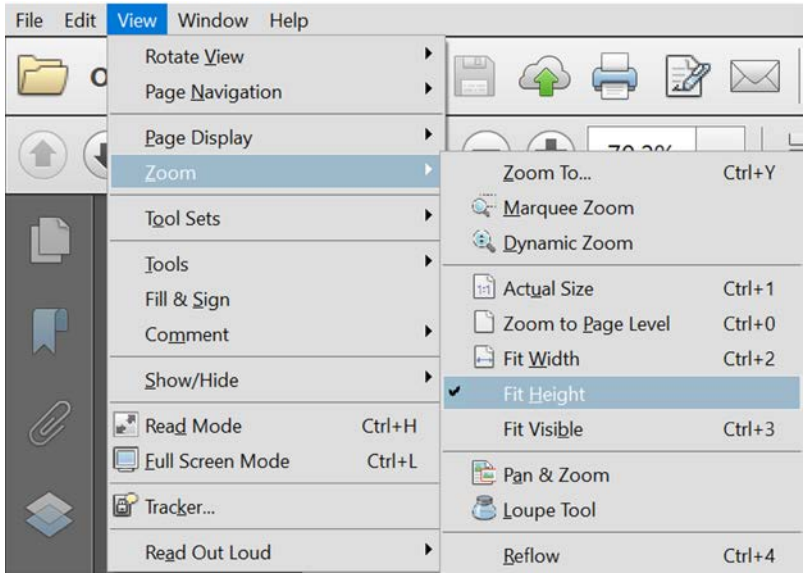
Prism 8 curve fitting to rise-and-fall to baseline exponential equation with baseline drift



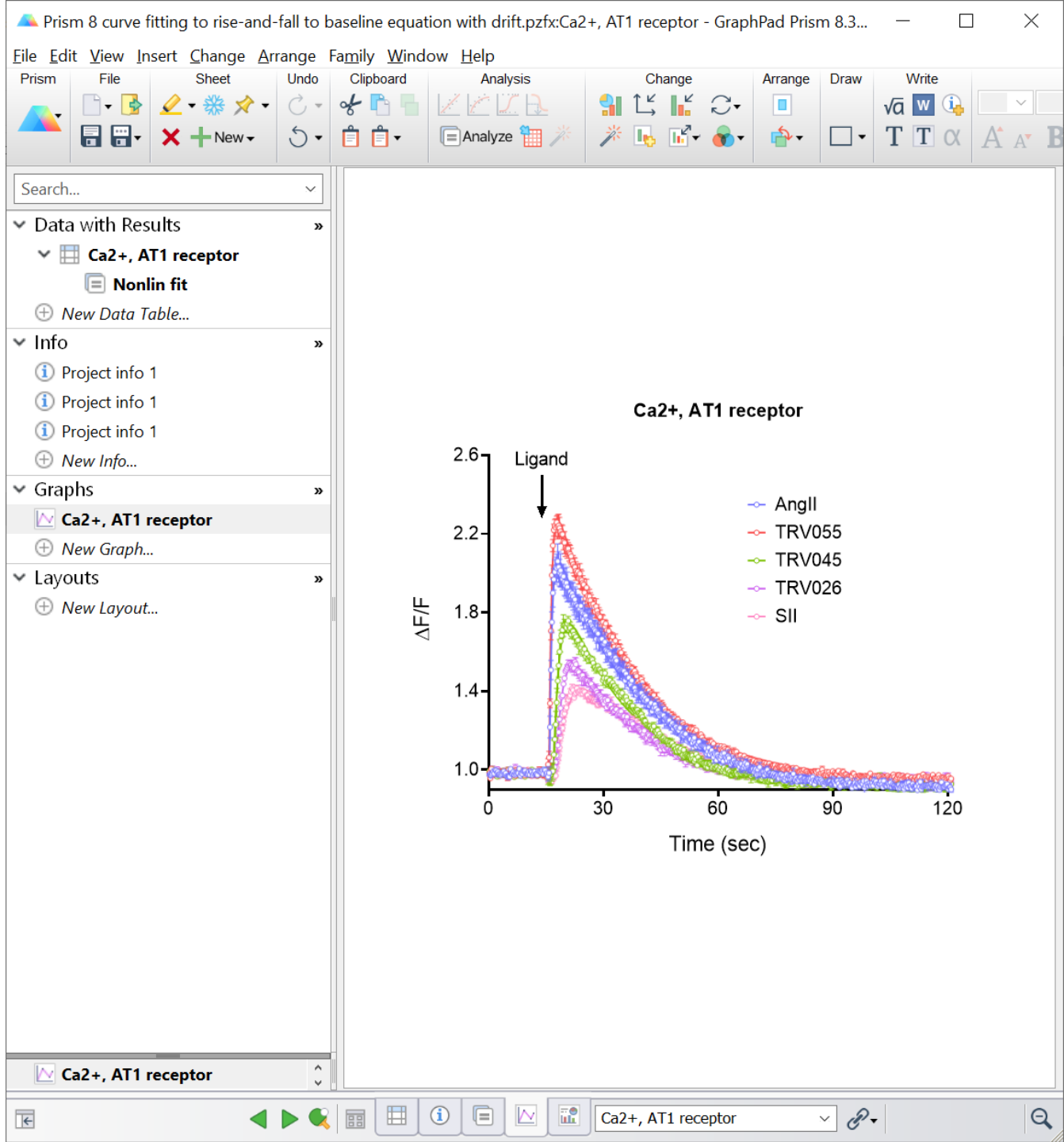
“Fit height” enables page flipping in PDF viewer

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

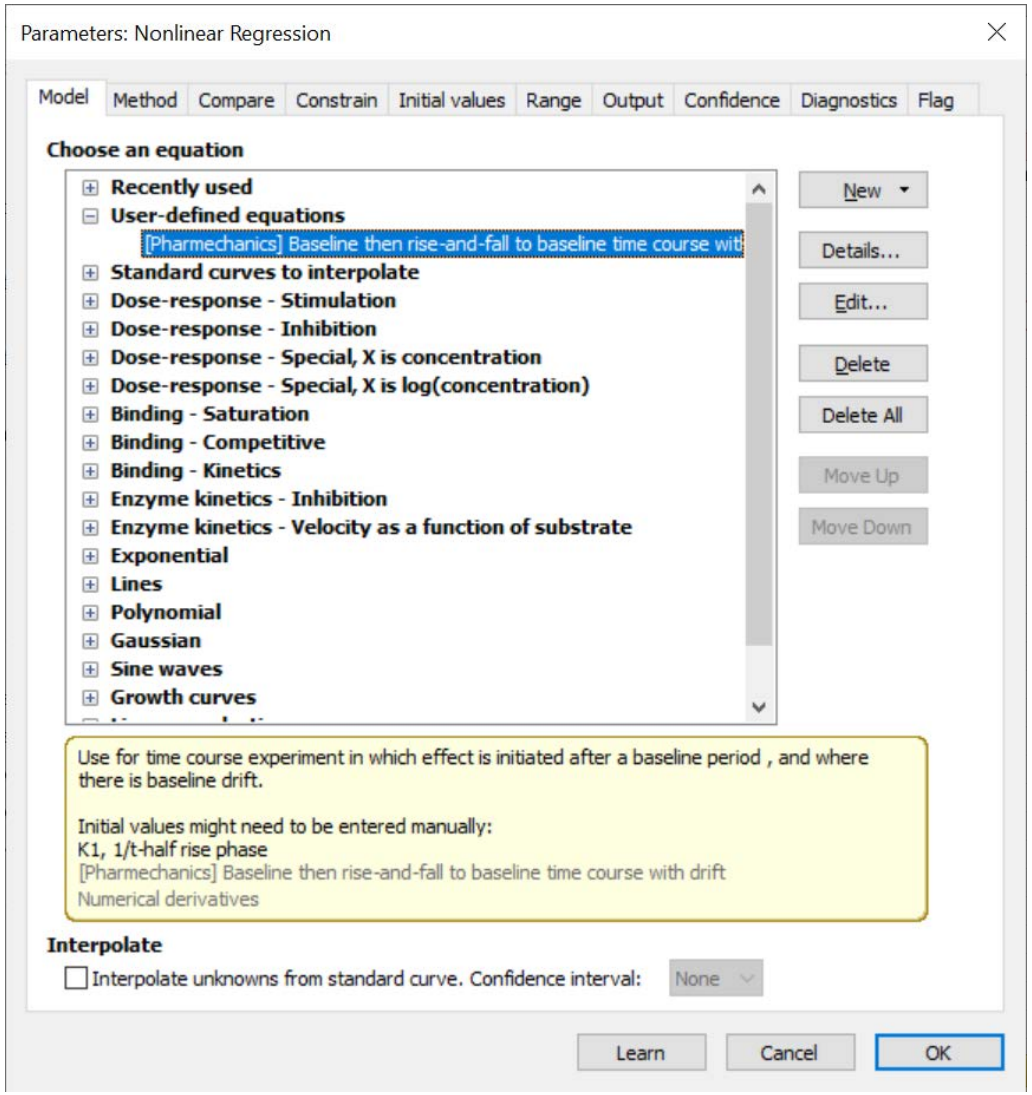
In this example there is baseline drift, i.e. the baseline falls slightly over time. This is handled using a modified equation.

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

The example will use is Ca2+ mobilization via the AT1 receptor, by five ligands – AngII and four synthetic ligands. A maximally-stimulating concentration was used (32 μM).



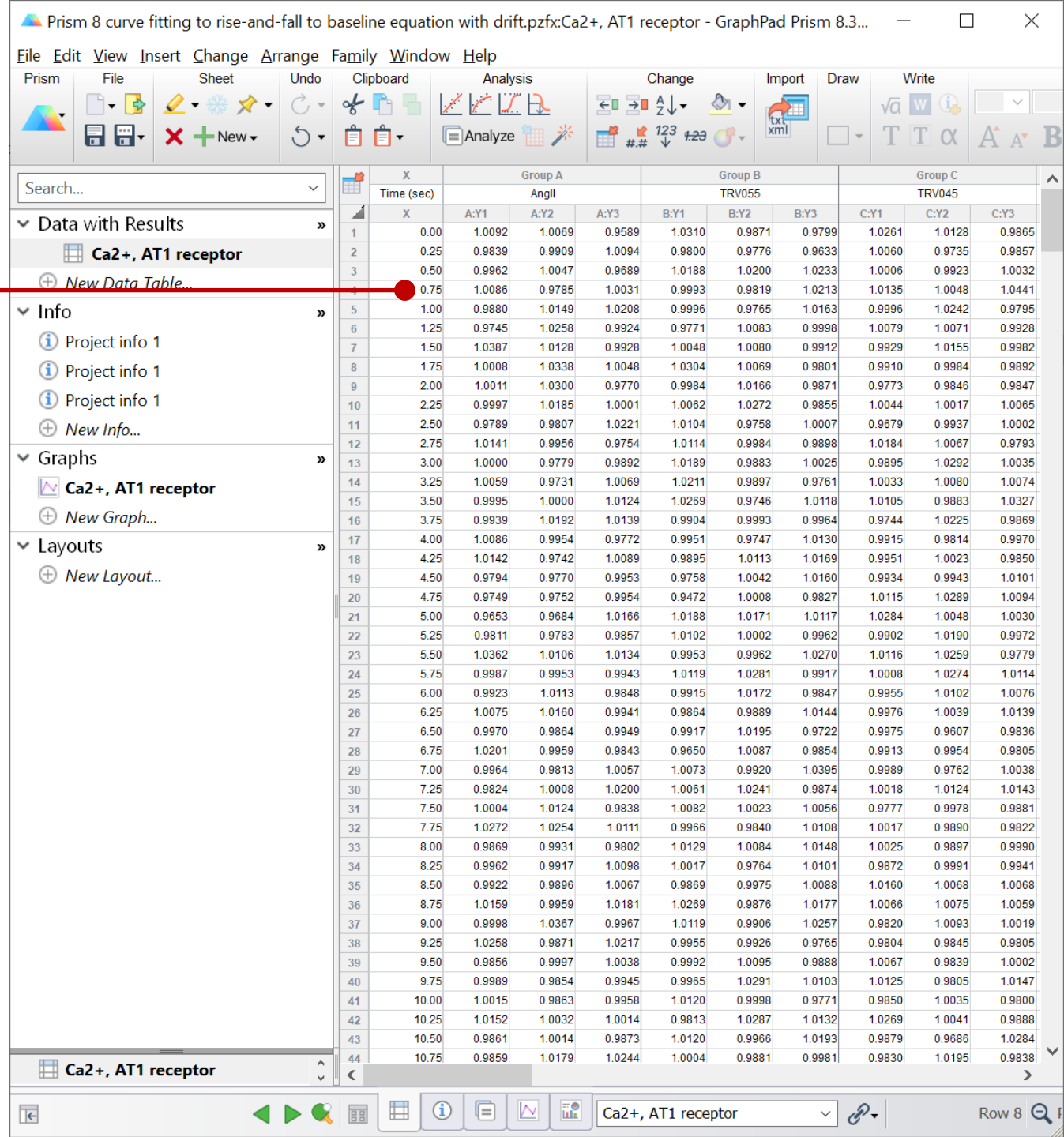
- First we need to load the equation.
- The rise-and-fall equation with drift is not a built-in equation in Prism.
- Instead it is loaded as a User-defined equation.
- This can be done easily from a template.
- This is shown starting on page 21.
- The equation is called: “[Pharmechanics] Baseline then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



Enter data into Prism data table

Notes

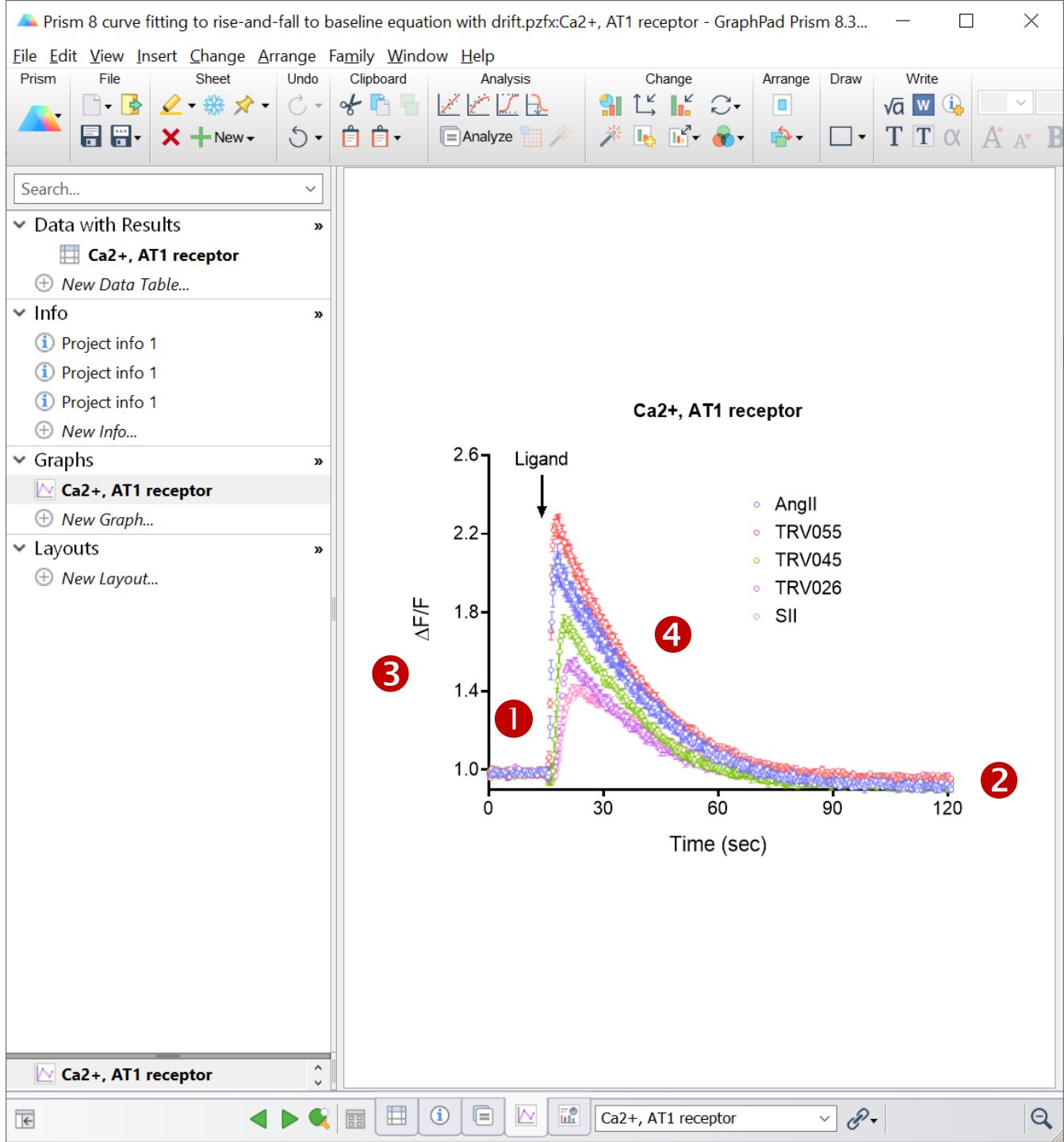
If you have technical replicates, enter each replicate, rather than just the mean.



Here is the graph of the data.

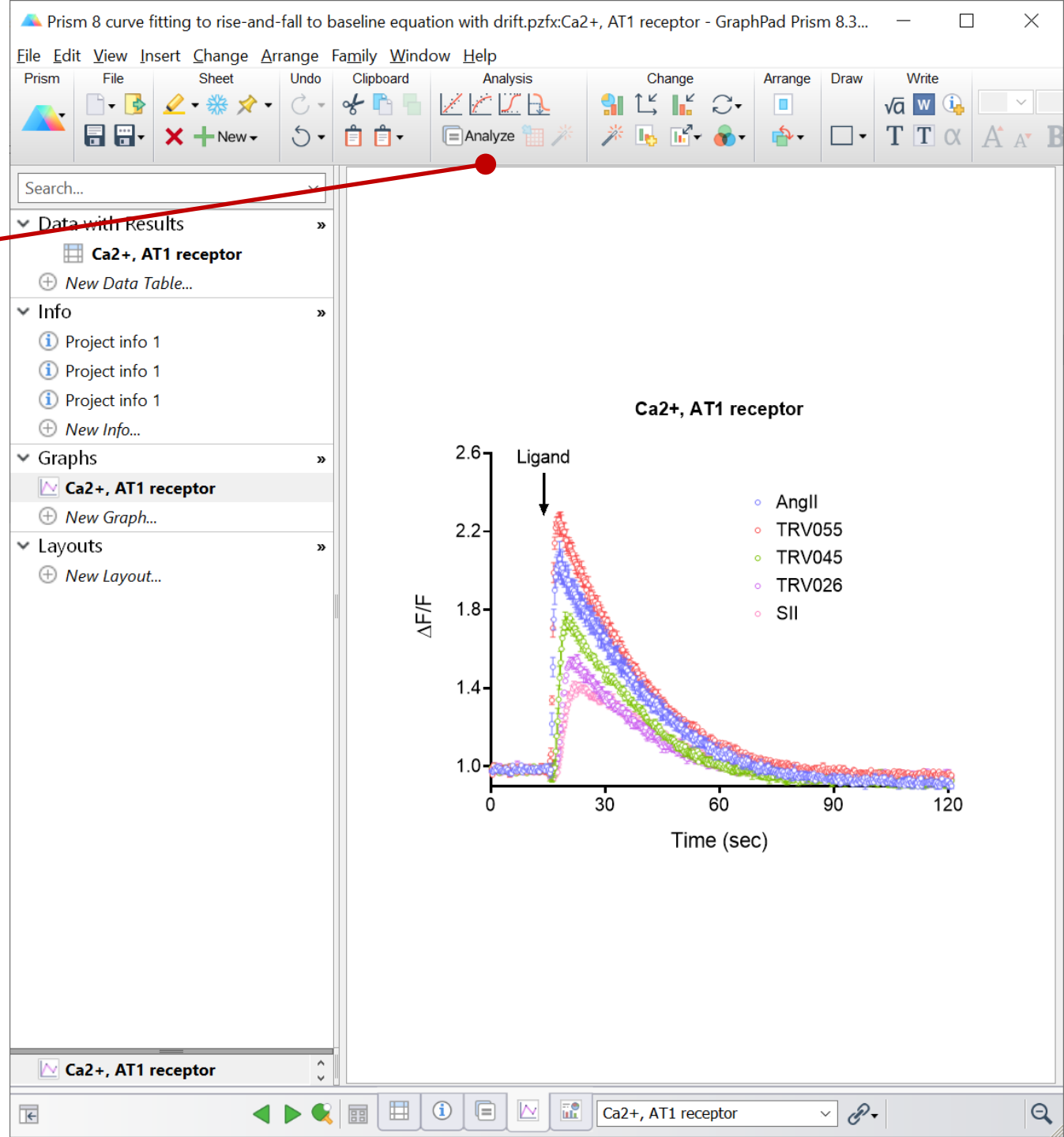
Notes

1. Lead-in phase before addition of ligand, where we are measuring the baseline signal.
2. Response at the end is slightly lower than response at the beginning. This indicates the baseline has drifted downwards.
3. Data are expressed as $\Delta F / F$. This is the fluorescence intensity after ligand addition divided by the average baseline fluorescence intensity before addition, calculated using a spreadsheet.
4. Error bars are SEM.



Now we will analyze the data.

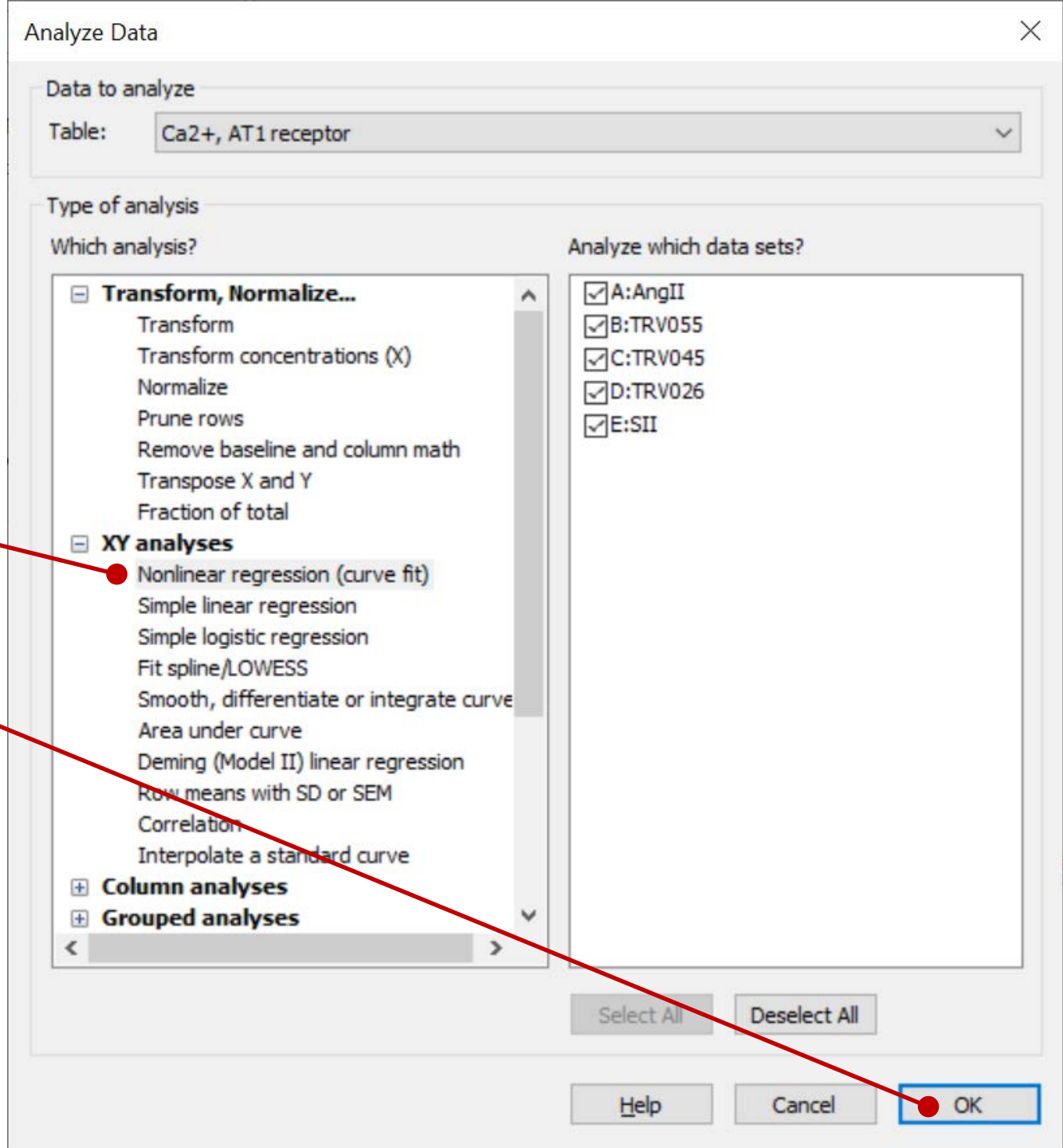
Click “Analyze”



This brings up the “Analyze Data” dialogue

Select “Nonlinear regression”

Click “OK”

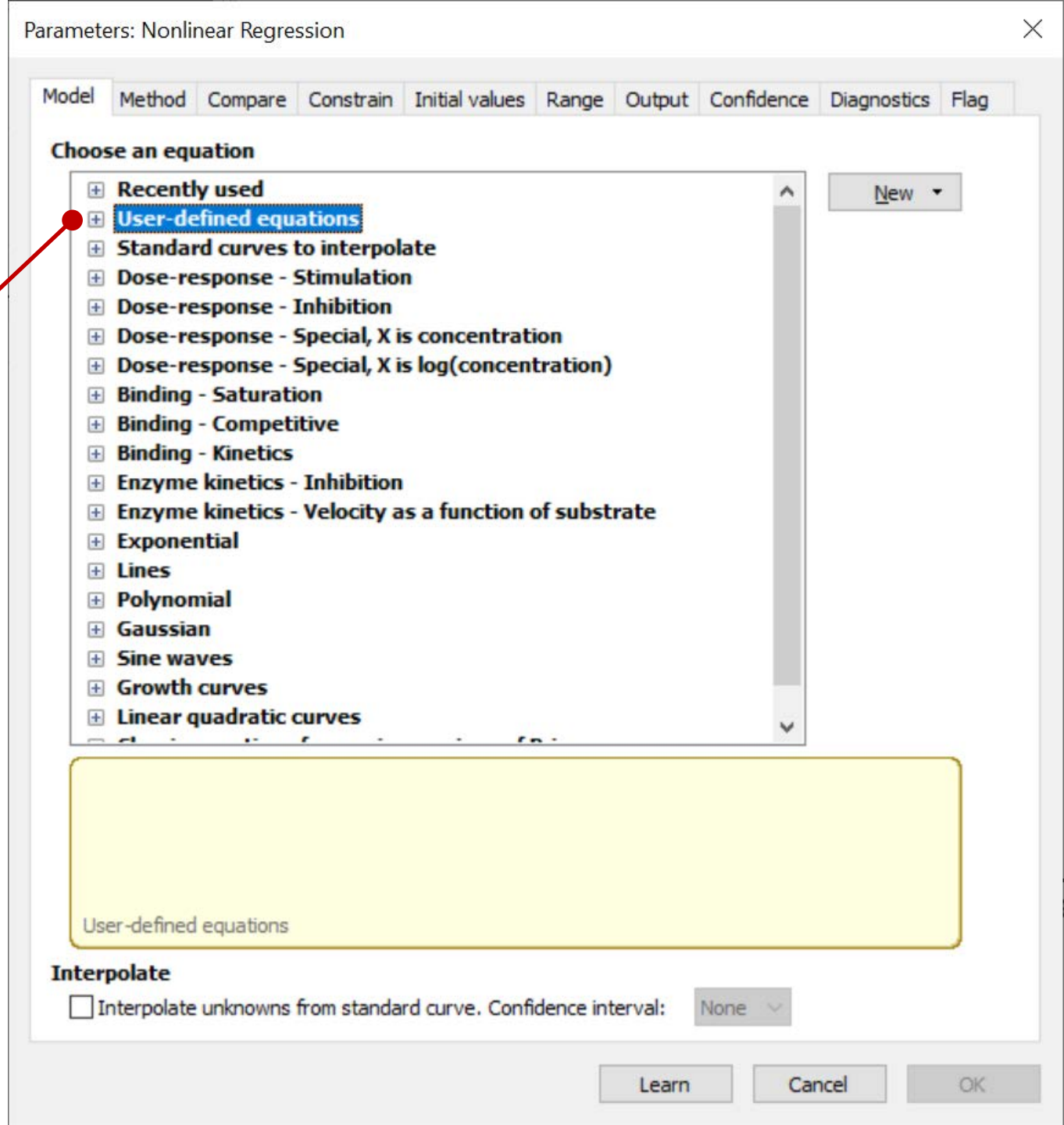


This brings up the “Parameters: Nonlinear Regression” dialogue.

Now we select the equation.

Click “User-defined equations” checkbox.

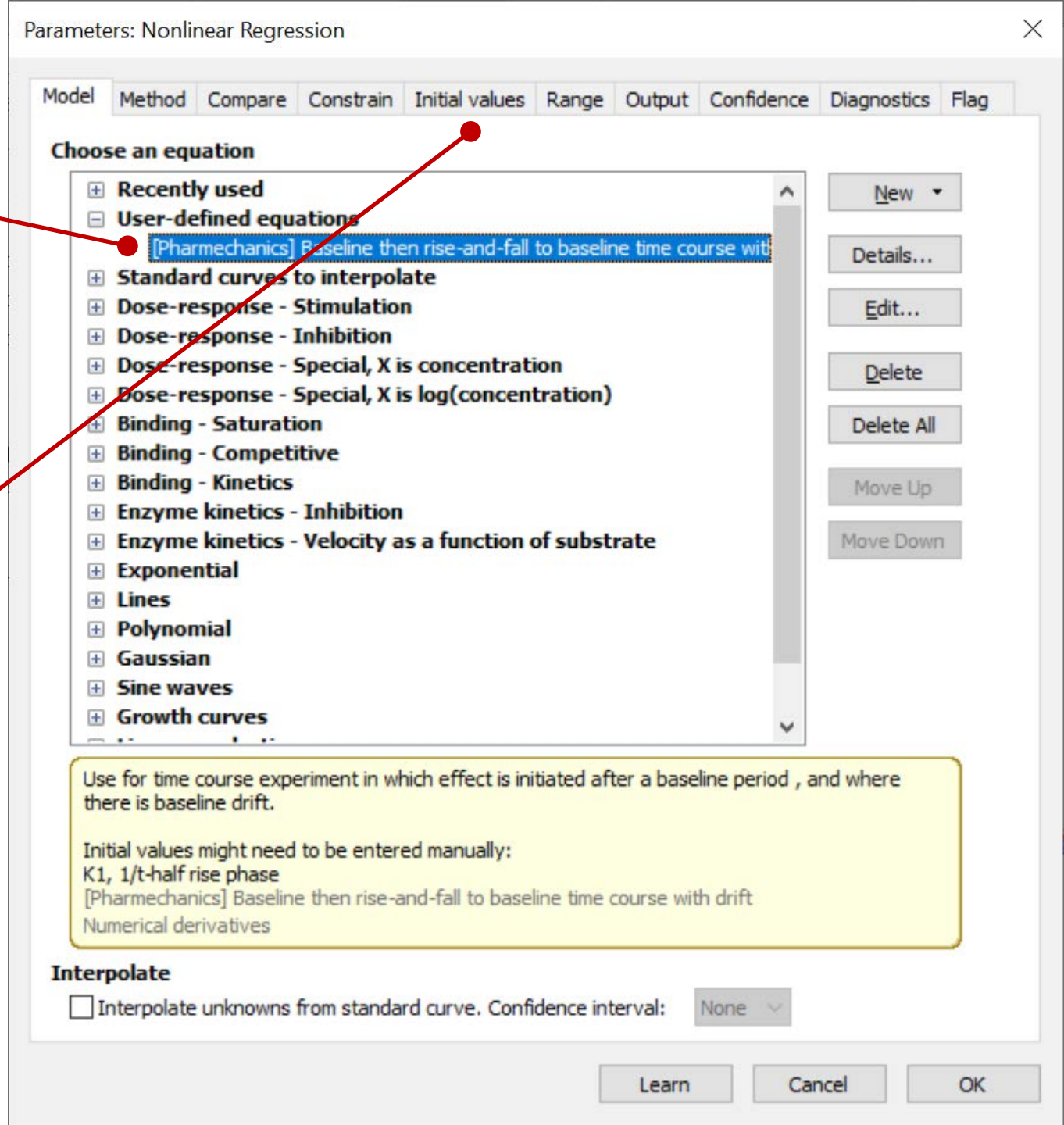
Note this will only appear if user-defined equations have been loaded. See page 21.



Select ““[Pharmechanics] Baseline then rise-and-fall to baseline with drift”

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

Click on the “Initial values” tab

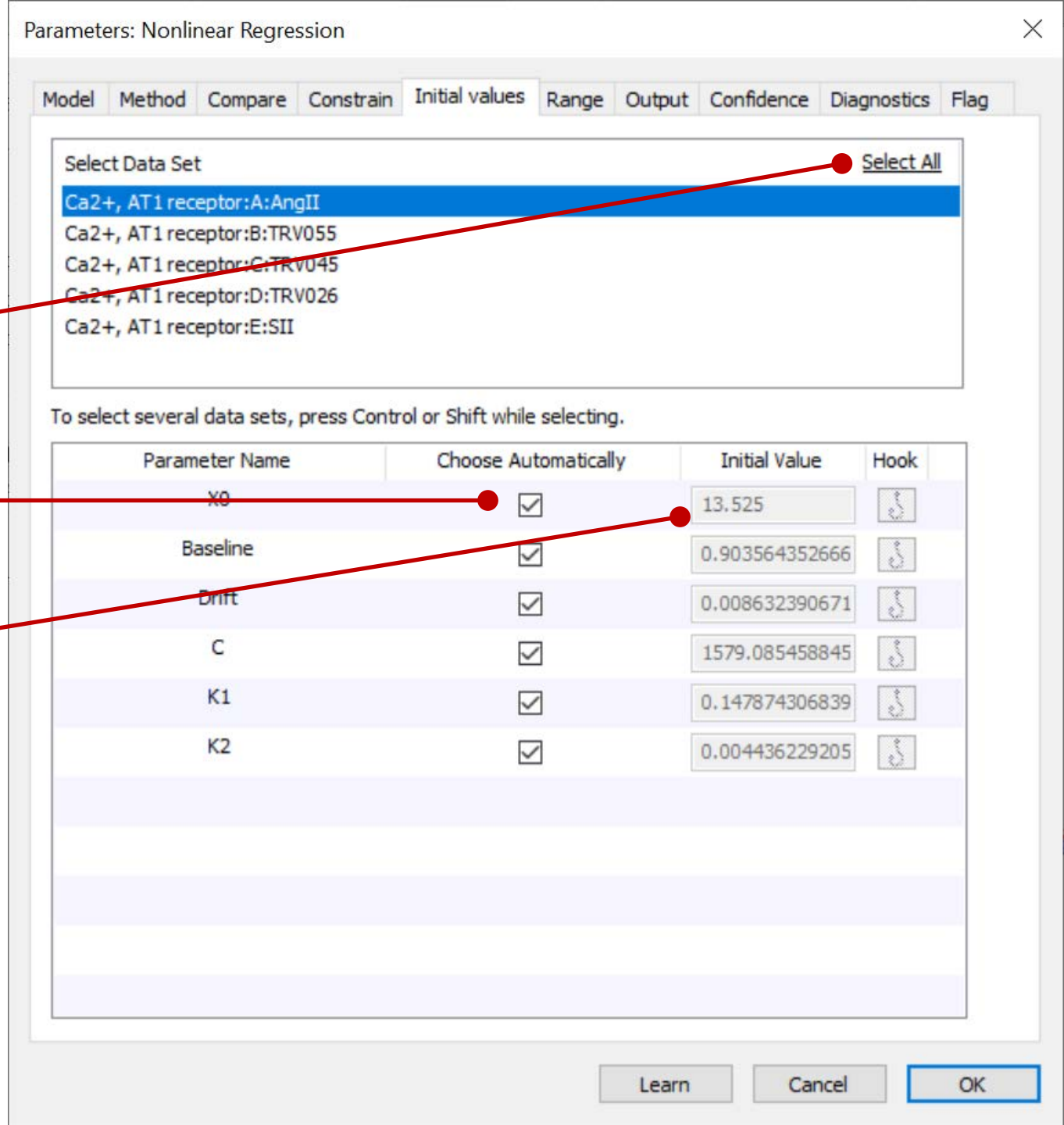


Here we recommend manually entering the X0 initial value. X0 is the time the signal starts to rise.

Click “Select All”

Uncheck “Choose Automatically” for X0

Enter the estimated X0 value
(from visual inspection of the graph)

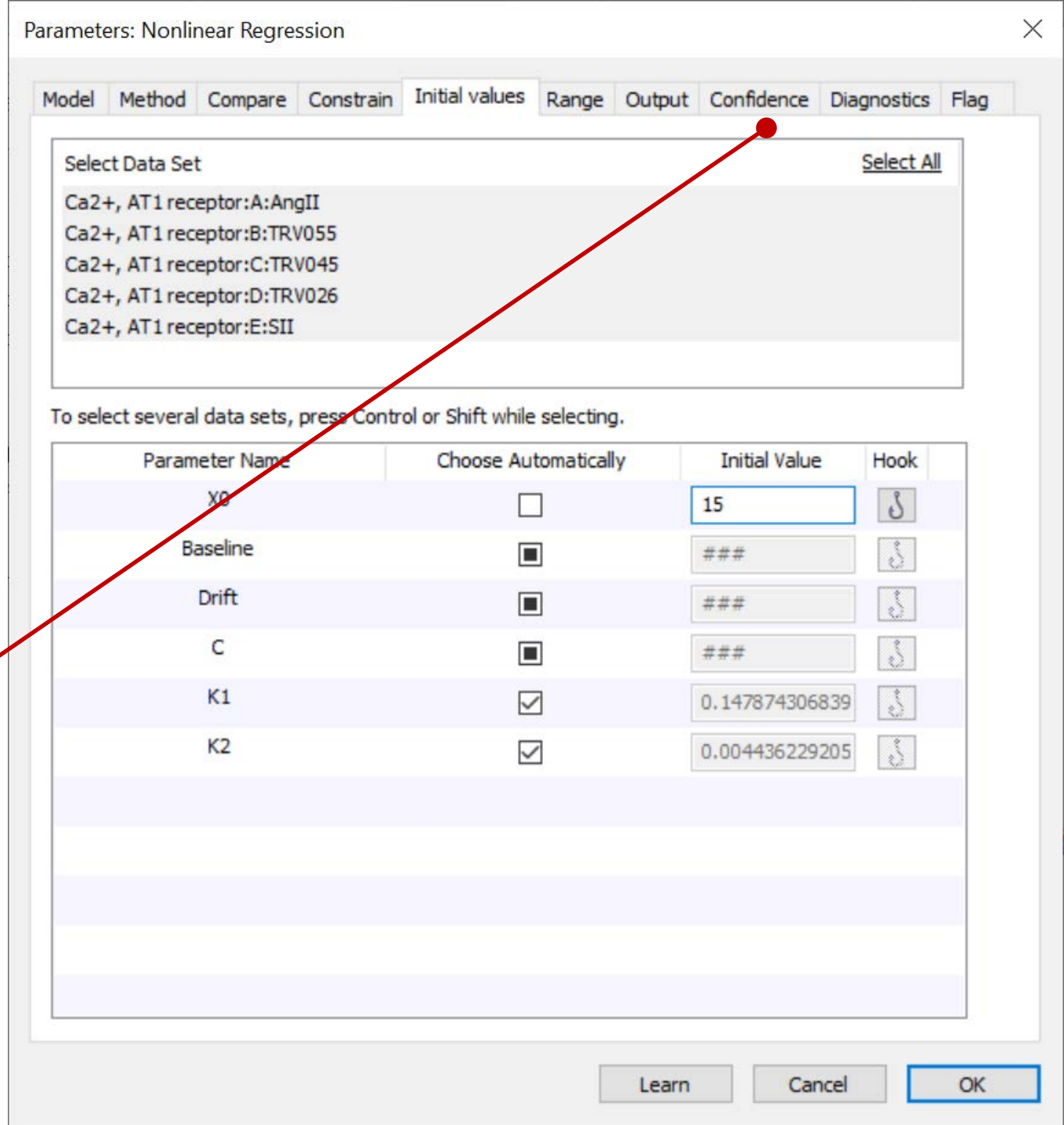


Now we are going to change a setting to make the analysis run efficiently.

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.

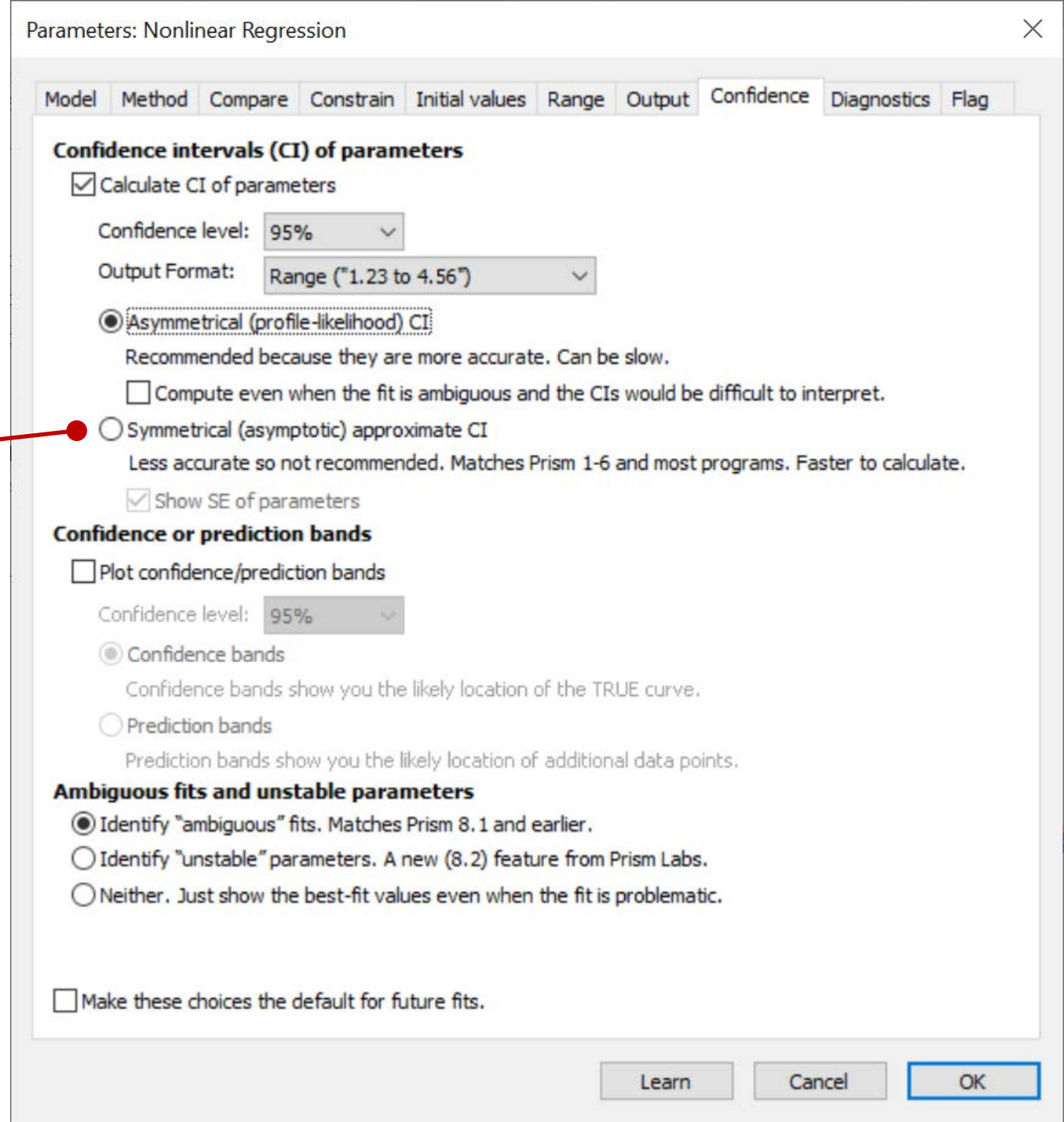
The rigorous method can be turned off to make the analysis run faster.

Click on the “Confidence” tab

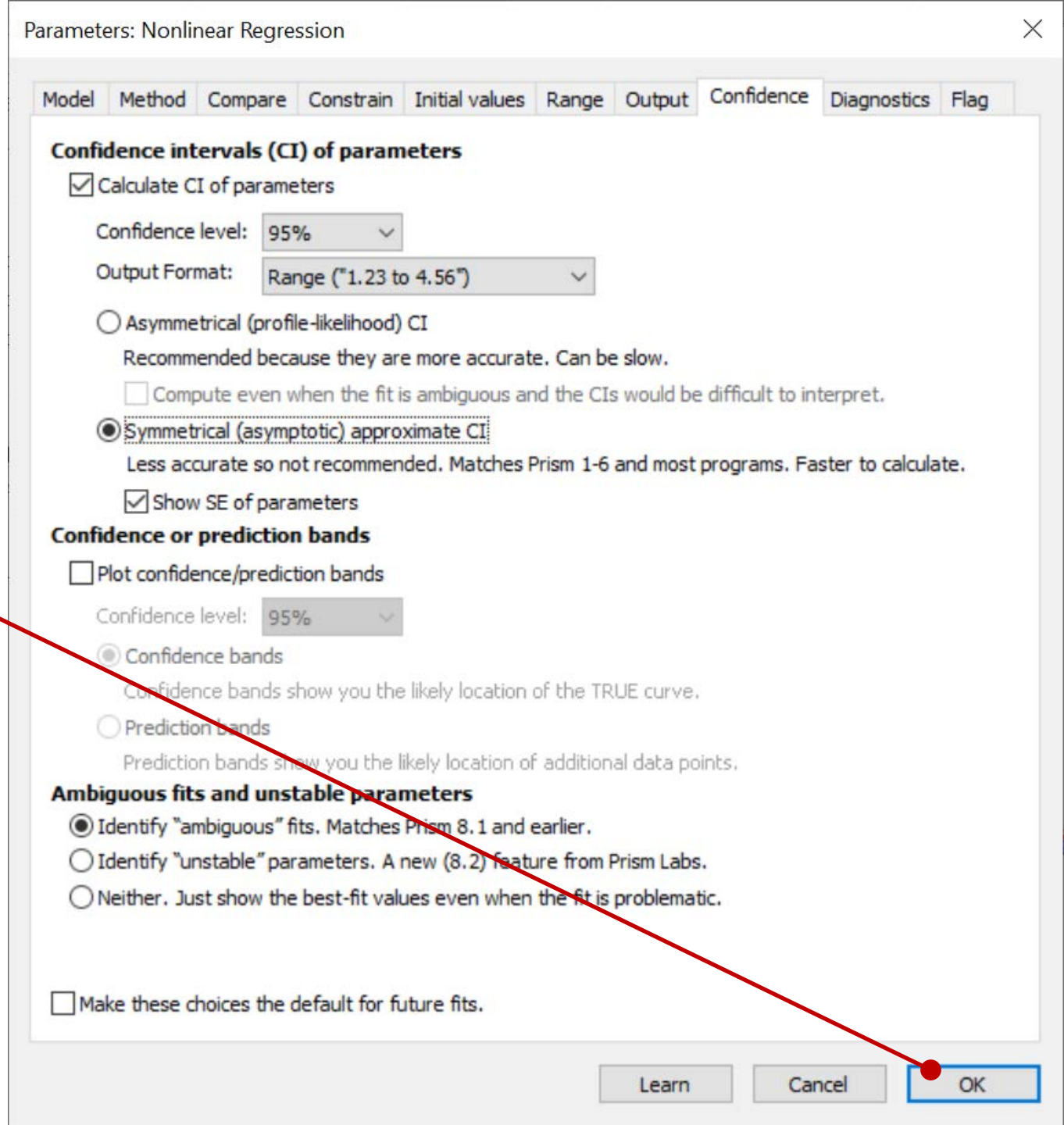


“Asymmetrical” is the rigorous error calculation method

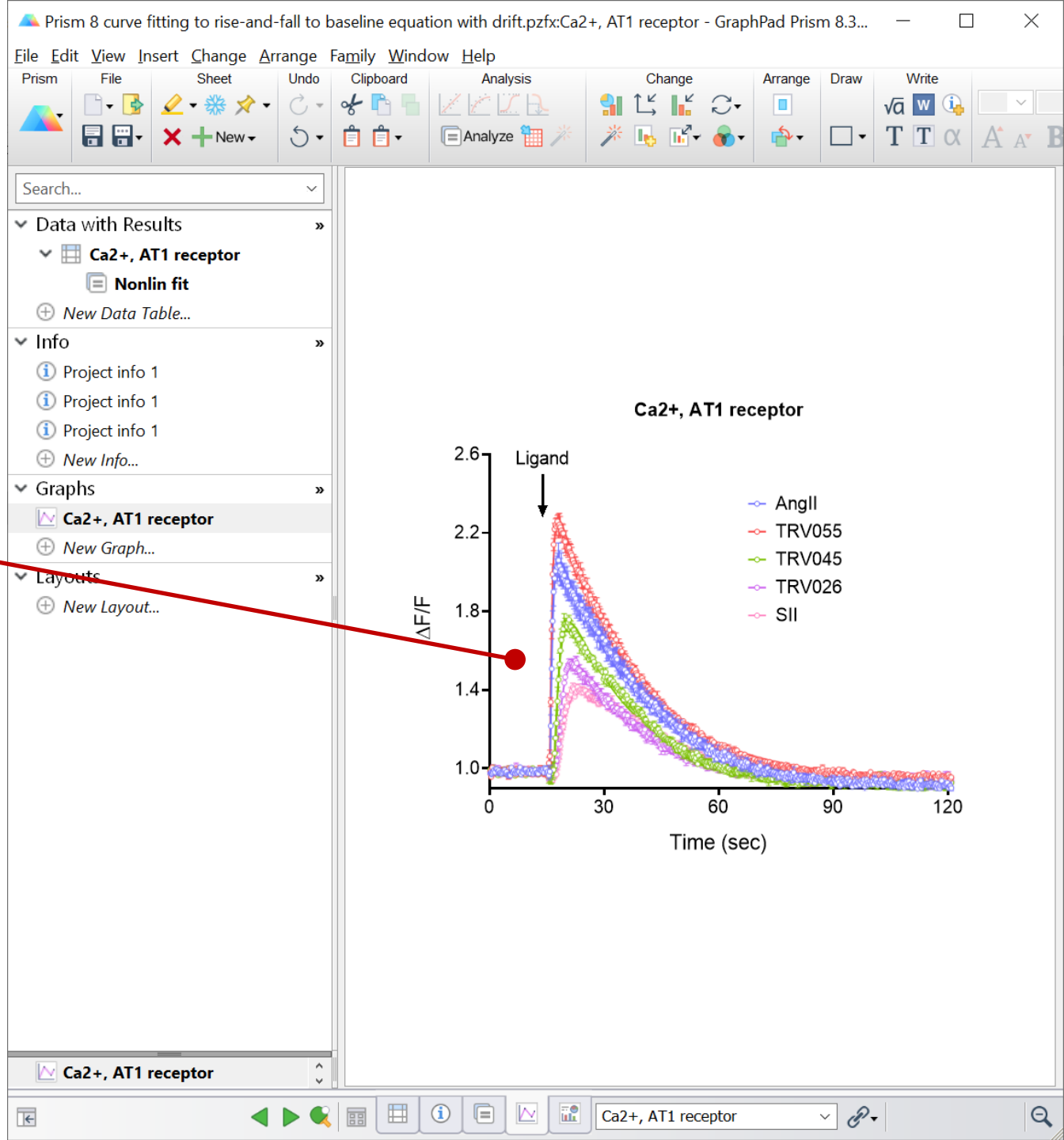
To turn it off, click the “Symmetrical” radio button



Click “OK” to run the analysis

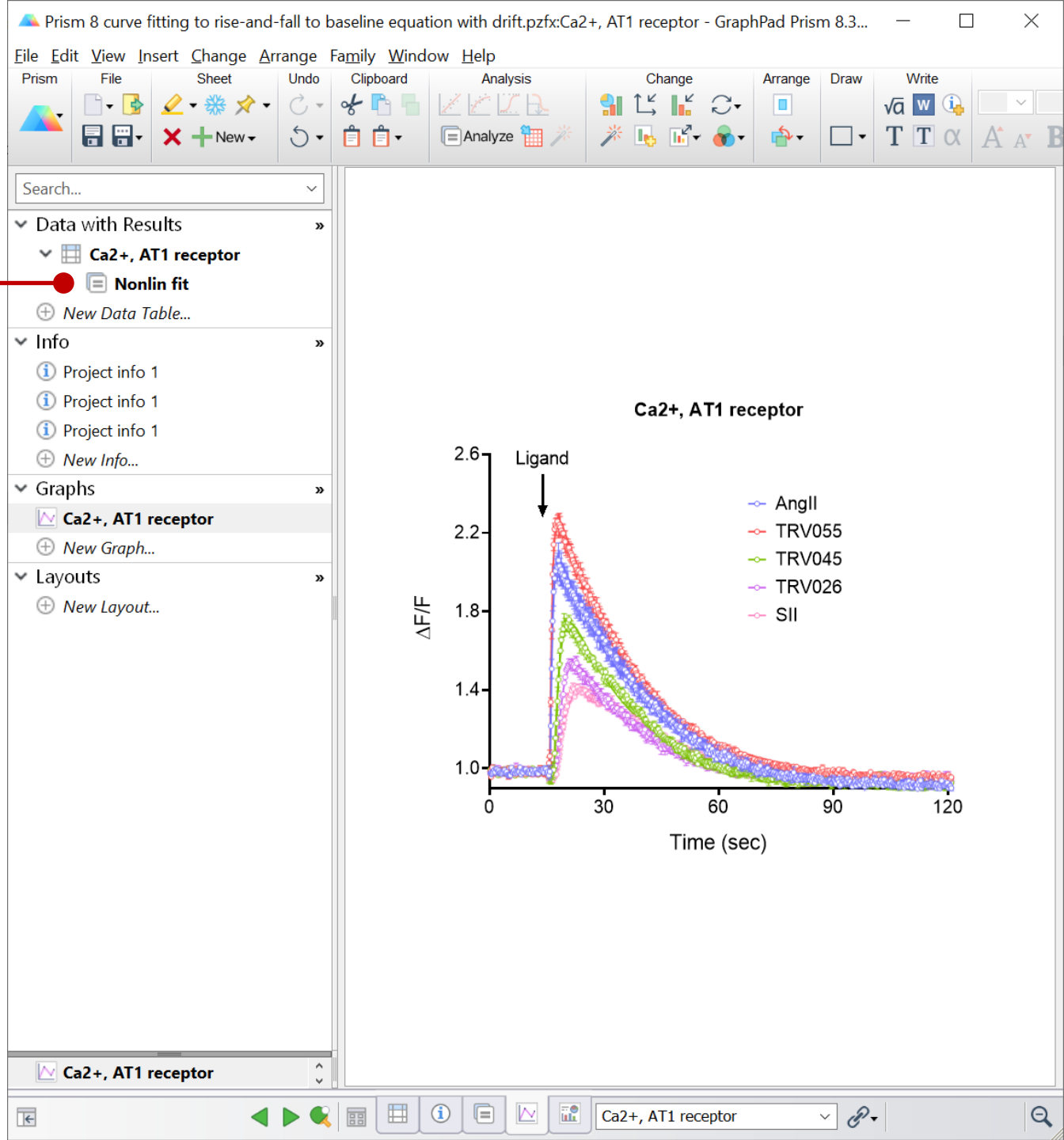


These are the fitted curves



Reviewing the results

Click “Nonlin fit” to bring up the results table



Prism 8 curve fitting to rise-and-fall to baseline equation with drift.pzfx:Nonlin fit of Ca2+, AT1 receptor - GraphPad Prism 8.3.0 (538)

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Search...

Data with Results
- Ca2+, AT1 receptor
  - Nonlin fit
- New Data Table...

Info
- Project info 1
- Project info 1
- Project info 1
- New Info...

Graphs
- Ca2+, AT1 receptor
- New Graph...

Layouts
- New Layout...

Nonlin fit

Table of results

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

Nonlin fit
Table of results

		A	B	C	D	E	F
		AngII	TRV055	TRV045	TRV026	SII	
1	**[Pharmechanics] Baseline then rise-and-fall to baseline time course with drift**						
2	**Best-fit values**						
3	X0	15.6	15.5	16.6	17.2	17.1	
4	Baseline	0.994	0.996	0.995	1.00	0.997	
		-0.000724	-0.000379	-0.000569	-0.000339	-0.000463	
		1.88	2.19	0.625	0.373	0.176	
		1.54	1.52	0.638	0.503	0.269	
		0.0475	0.0492	0.0589	0.0638	0.0548	
		0.449	0.457	1.09	1.38	2.57	
		14.6	14.1	11.8	10.9	12.6	
11	**Std. Error**						
12	X0	0.0201	0.00774	0.0281	0.0287	0.0447	
13	Baseline	0.00342	0.00241	0.00265	0.00190	0.00174	
14	Drift	3.40e-005	2.40e-005	2.71e-005	1.96e-005	1.77e-005	
15	C	0.0813	0.0449	0.0179	0.00932	0.00386	
16	K1	0.0694	0.0325	0.0207	0.0149	0.00801	
17	K2	0.000452	0.000280	0.000703	0.000824	0.000890	
18	**95% CI (asymptotic)**						
19	X0	15.6 to 15.6	15.5 to 15.5	16.6 to 16.7	17.1 to 17.2	17.1 to 17.2	
20	Baseline	0.988 to 1.00	0.991 to 1.00	0.990 to 1.00	0.996 to 1.00	0.994 to 1.00	
21	Drift	-0.000791 to -0.000658	-0.000426 to -0.000332	-0.000623 to -0.000516	-0.000377 to -0.000300	-0.000498 to -0.000428	
22	C	1.72 to 2.04	2.10 to 2.28	0.590 to 0.661	0.355 to 0.392	0.168 to 0.183	
23	K1	1.41 to 1.68	1.45 to 1.58	0.598 to 0.679	0.474 to 0.532	0.254 to 0.285	
24	K2	0.0466 to 0.0484	0.0487 to 0.0498	0.0575 to 0.0603	0.0621 to 0.0654	0.0531 to 0.0566	
25	Half-time K1	0.413 to 0.493	0.438 to 0.477	1.02 to 1.16	1.30 to 1.46	2.43 to 2.73	
26	Half-time K2	14.3 to 14.9	13.9 to 14.2	11.5 to 12.0	10.6 to 11.2	12.3 to 13.1	
27	**Goodness of Fit**						
28	Degrees of Freedom	1620	1620	1620	1620	1620	
29	R squared	0.977	0.991	0.972	0.971	0.971	
30	Sum of Squares	3.25	1.60	2.09	1.11	0.905	
31	Sy.x	0.0448	0.0315	0.0359	0.0262	0.0236	
32	**Constraints**						
33	C	C > 0	C > 0	C > 0	C > 0	C > 0	
34	K2	K2 > 0	K2 > 0	K2 > 0	K2 > 0	K2 > 0	
35							
36	**Number of points**						

Nonlin fit of Ca2+, AT1 receptc Table of results

Prism 8 curve fitting to rise-and-fall to baseline equation with drift.pzfx:Nonlin fit of Ca2+, AT1 receptor - GraphPad Prism 8.3.0 (538)

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text Export Print Send LA Help

Search...

- Data with Results
  - Ca2+, AT1 receptor
    - Nonlin fit
  - New Data Table...
- Info
  - Project info 1
  - Project info 1
  - Project info 1
  - New Info...
- Graphs
  - Ca2+, AT1 receptor
  - New Graph...
- Layouts
  - New Layout...

Nonlin fit

Table of results

Nonlin fit
Table of results

		A	B	C	D	E	F
		AngII	TRV055	TRV045	TRV026	SII	
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6	C	1.88	2.19	0.625	0.373	0.176	
7	K1	1.54	1.52	0.638	0.503	0.269	
		0.0475	0.0492	0.0589	0.0638	0.0548	
		0.449	0.457	1.09	1.38	2.57	
		14.6	14.1	11.8	10.9	12.6	
		0.0201	0.00774	0.0281	0.0287	0.0447	
		0.00342	0.00241	0.00265	0.00190	0.00174	
14	Drift	3.40e-005	2.40e-005	2.71e-005	1.96e-005	1.77e-005	
15	C	0.0813	0.0449	0.0179	0.00932	0.00386	
16	K1	0.0694	0.0325	0.0207	0.0149	0.00801	
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18	**95% CI (asymptotic)**						
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20	Baseline	0.988 to 1.00	0.991 to 1.00	0.990 to 1.00	0.996 to 1.00	0.994 to 1.00	
21	Drift	-0.000791 to -0.000658	-0.000426 to -0.000332	-0.000623 to -0.000516	-0.000377 to -0.000300	-0.000498 to -0.000428	
22	C	1.72 to 2.04	2.10 to 2.28	0.590 to 0.661	0.355 to 0.392	0.168 to 0.183	
23	K1	1.41 to 1.68	1.45 to 1.58	0.598 to 0.679	0.474 to 0.532	0.254 to 0.285	
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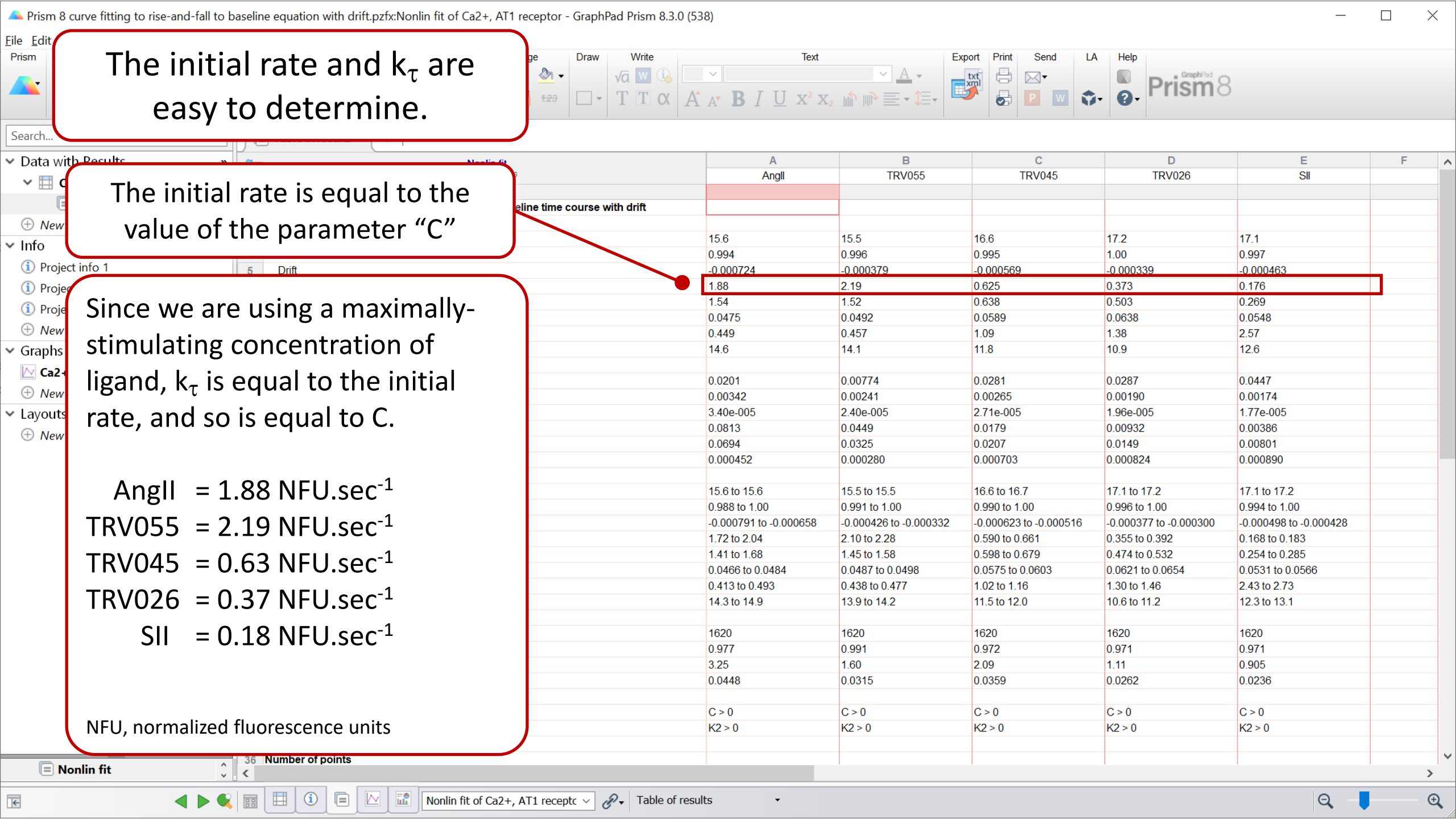
25	Half-time K1	0.413 to 0.493	0.438 to 0.477	1.02 to 1.16	1.30 to 1.46	2.43 to 2.73	
26	Half-time K2	14.3 to 14.9	13.9 to 14.2	11.5 to 12.0	10.6 to 11.2	12.3 to 13.1	
27	**Goodness of Fit**						
28	Degrees of Freedom	1620	1620	1620	1620	1620	
29	R squared	0.977	0.991	0.972	0.971	0.971	
30	Sum of Squares	3.25	1.60	2.09	1.11	0.905	
31	Sy.x	0.0448	0.0315	0.0359	0.0262	0.0236	
32	**Constraints**						
33	C	C > 0	C > 0	C > 0	C > 0	C > 0	
34	K2	K2 > 0	K2 > 0	K2 > 0	K2 > 0	K2 > 0	
35							
36	**Number of points**						

Data are fit well by the equation:
R squared > 0.9

Nonlin fit of Ca2+, AT1 receptc | Table of results

Calculating the initial rate and k_τ


Prism 8 curve fitting to rise-and-fall to baseline equation with drift.pzfx:Nonlin fit of Ca2+, AT1 receptor - GraphPad Prism 8.3.0 (538)
The initial rate and k_τ are easy to determine.
The initial rate is equal to the value of the parameter "C"
Since we are using a maximally-stimulating concentration of ligand, k_τ is equal to the initial rate, and so is equal to C.
AngII = 1.88 NFU.sec^{-1}
TRV055 = 2.19 NFU.sec^{-1}
TRV045 = 0.63 NFU.sec^{-1}
TRV026 = 0.37 NFU.sec^{-1}
SII = 0.18 NFU.sec^{-1}
NFU, normalized fluorescence units
A AngII
B TRV055
C TRV045
D TRV026
E SII
Drift
Number of points
C > 0
K2 > 0

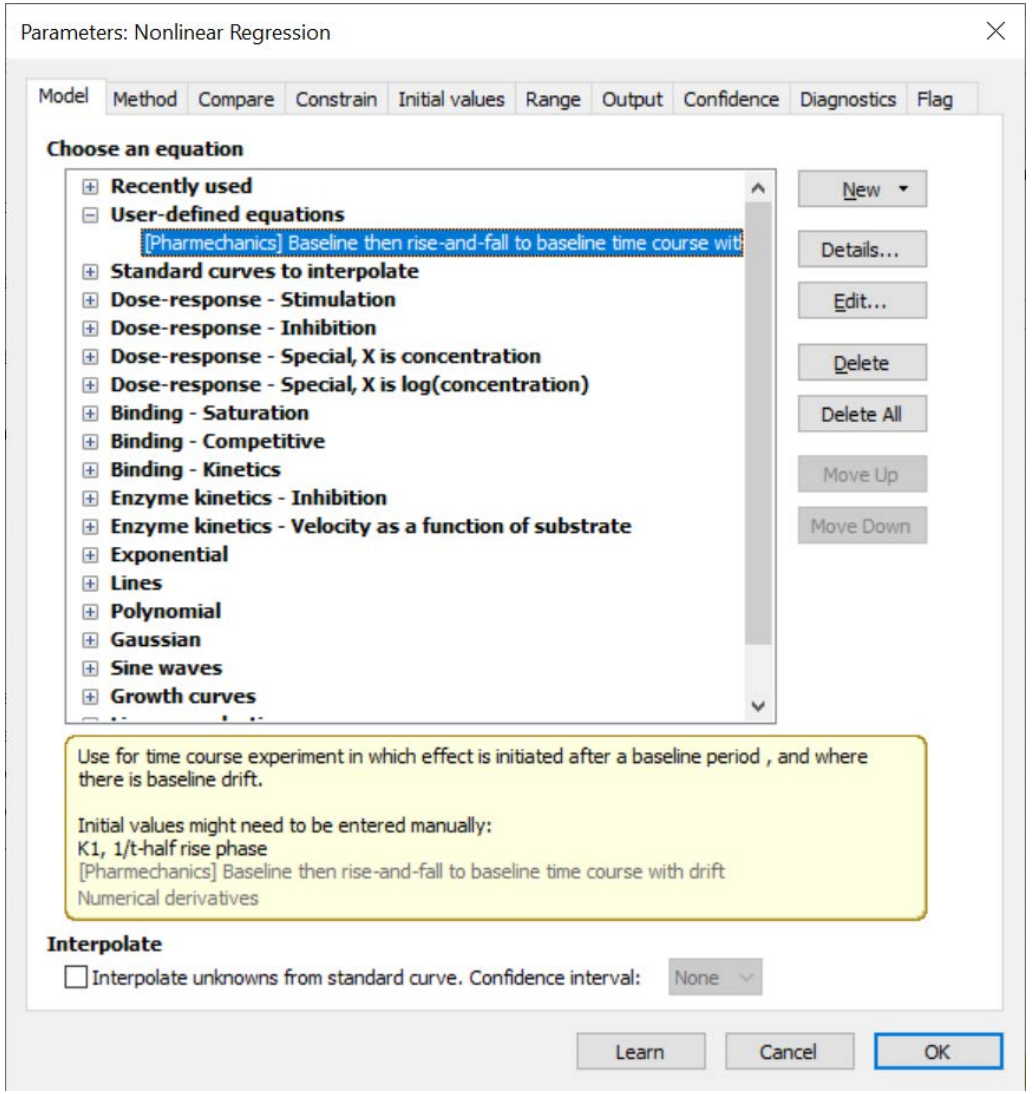
Loading equations into Prism from a file

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.

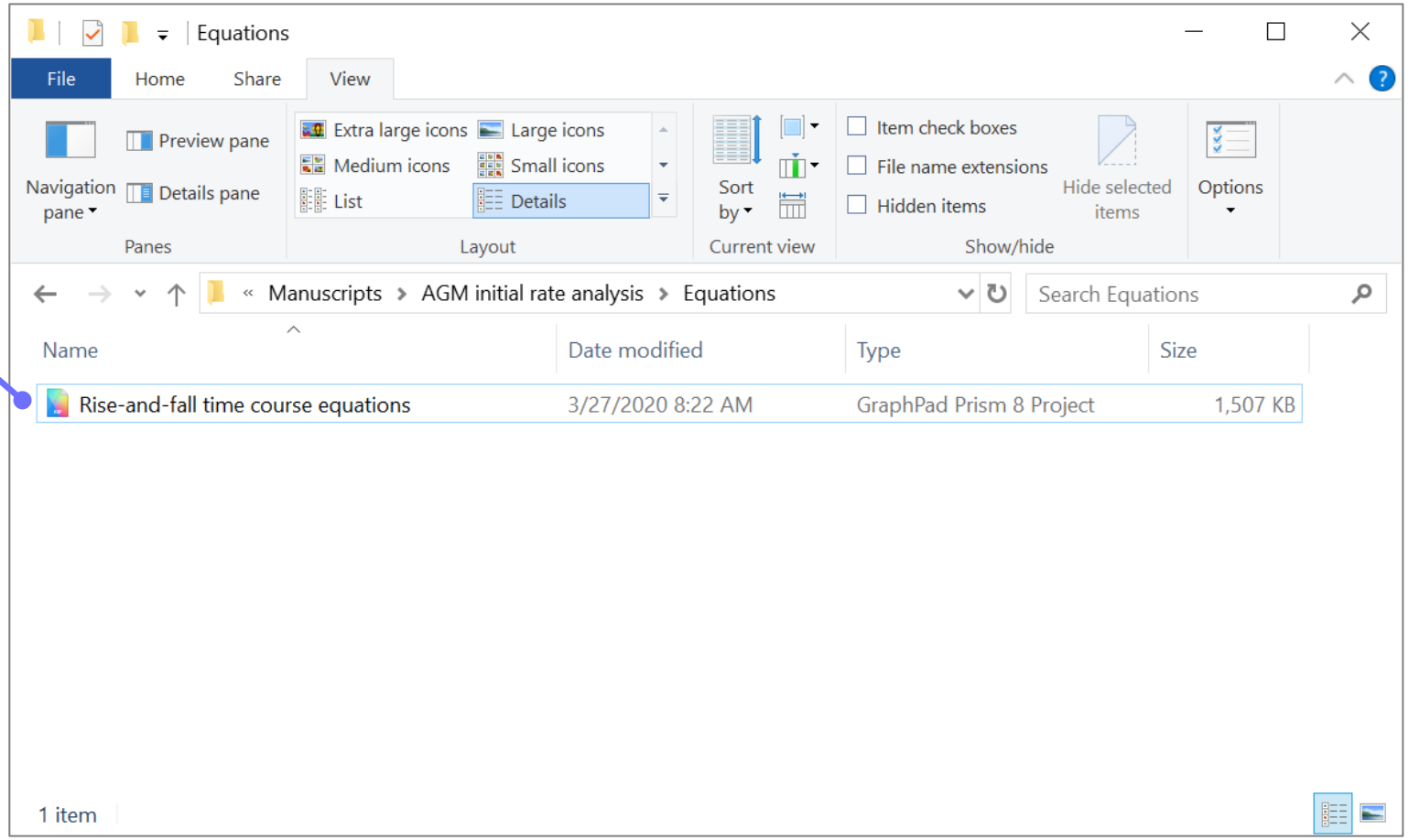
This avoids the need to write in the equation and all the fit settings.

This process is described in this presentation.



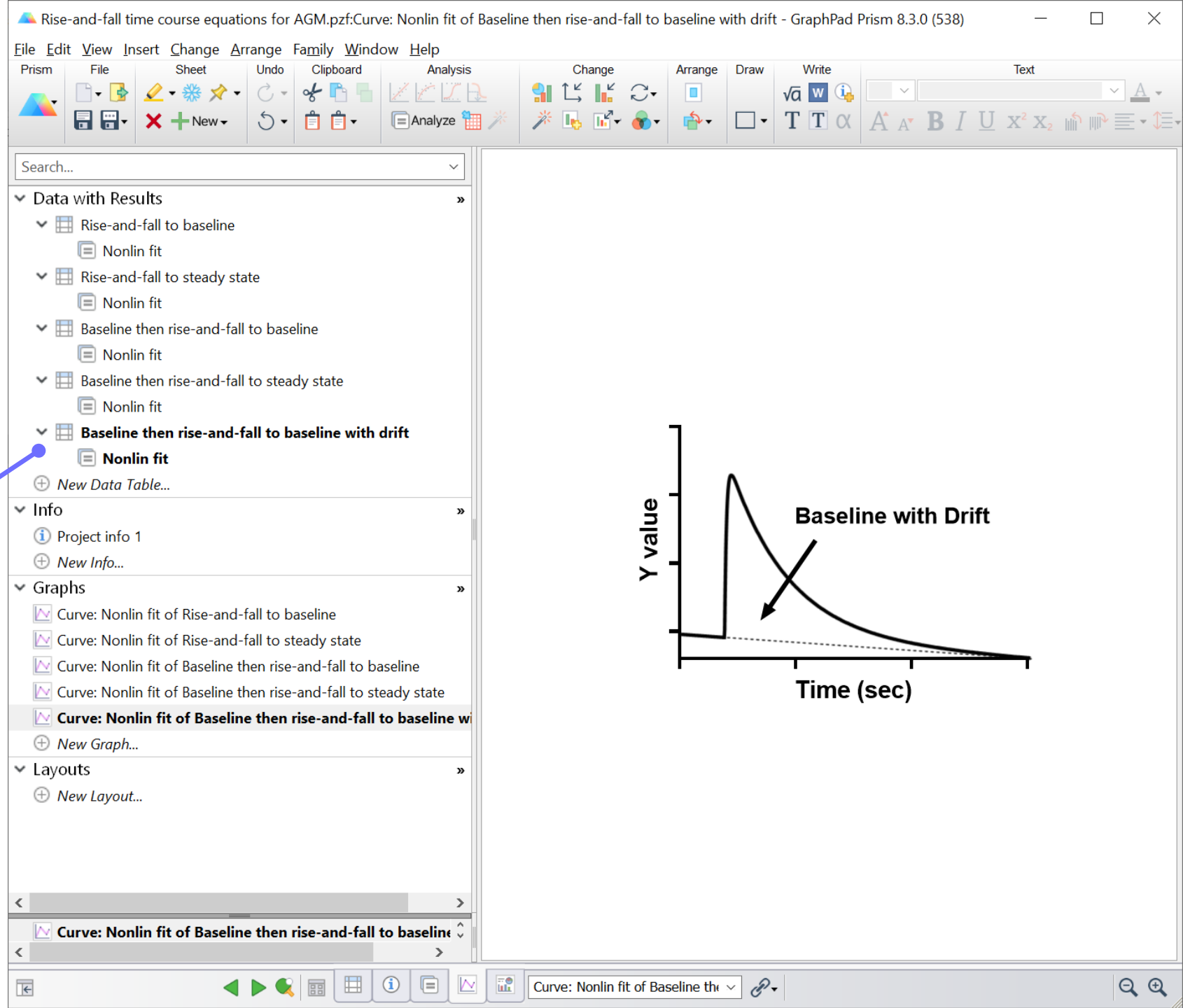
First, download the file containing the equations to your computer.

Then open it.

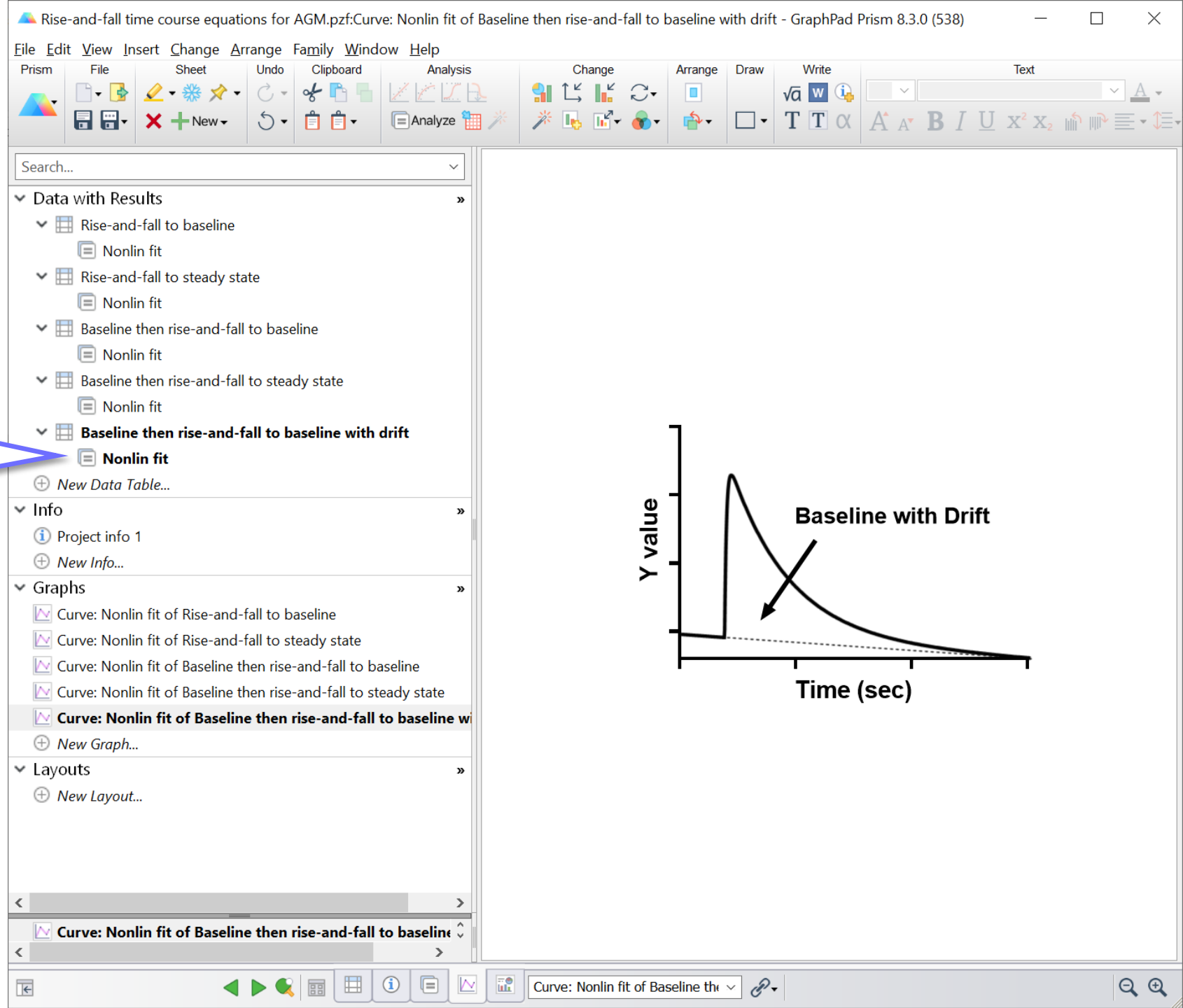


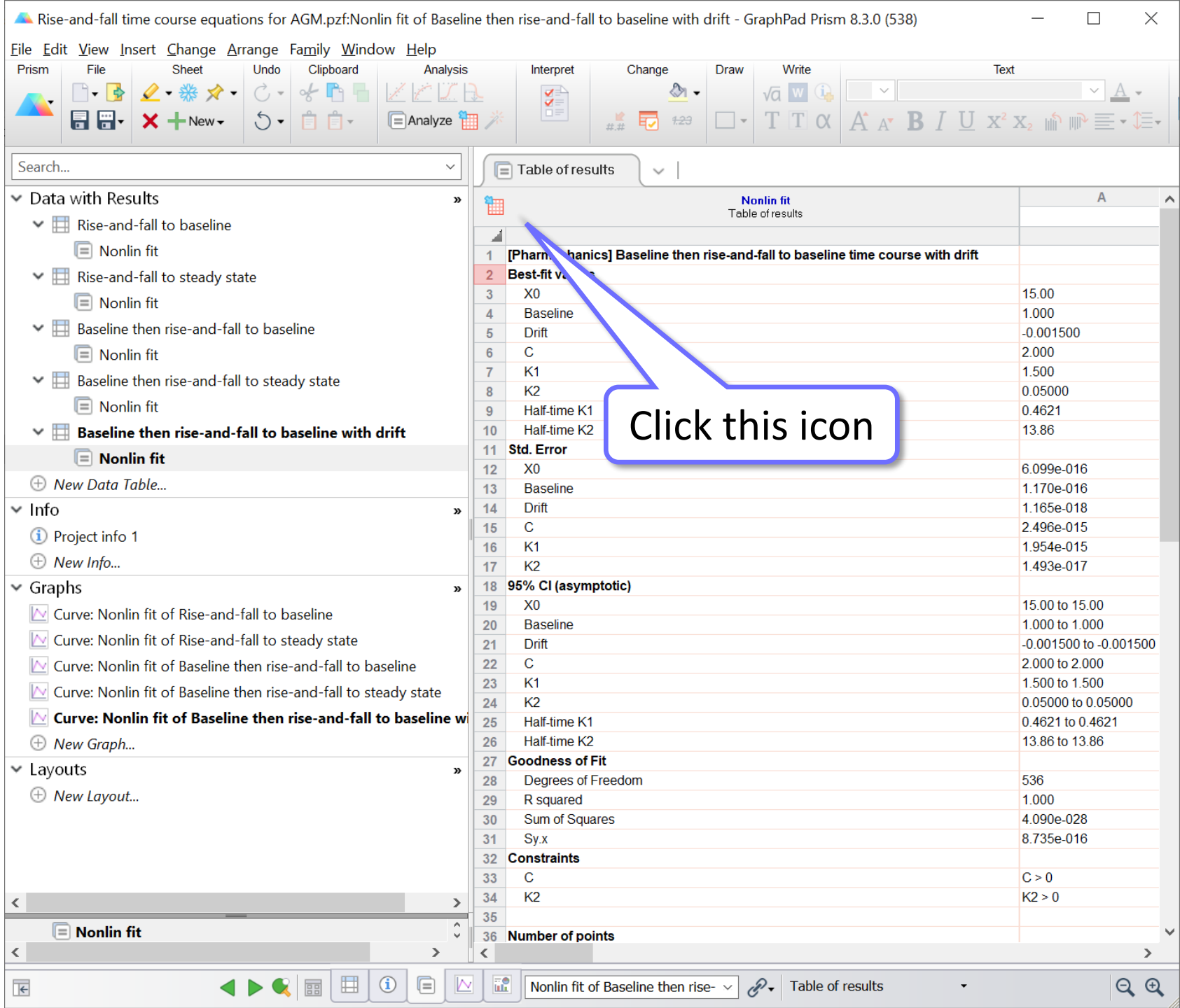
We are going to load the equation from the Results sheet.

The equation we need is called “Baseline then rise-and-fall to baseline with drift”

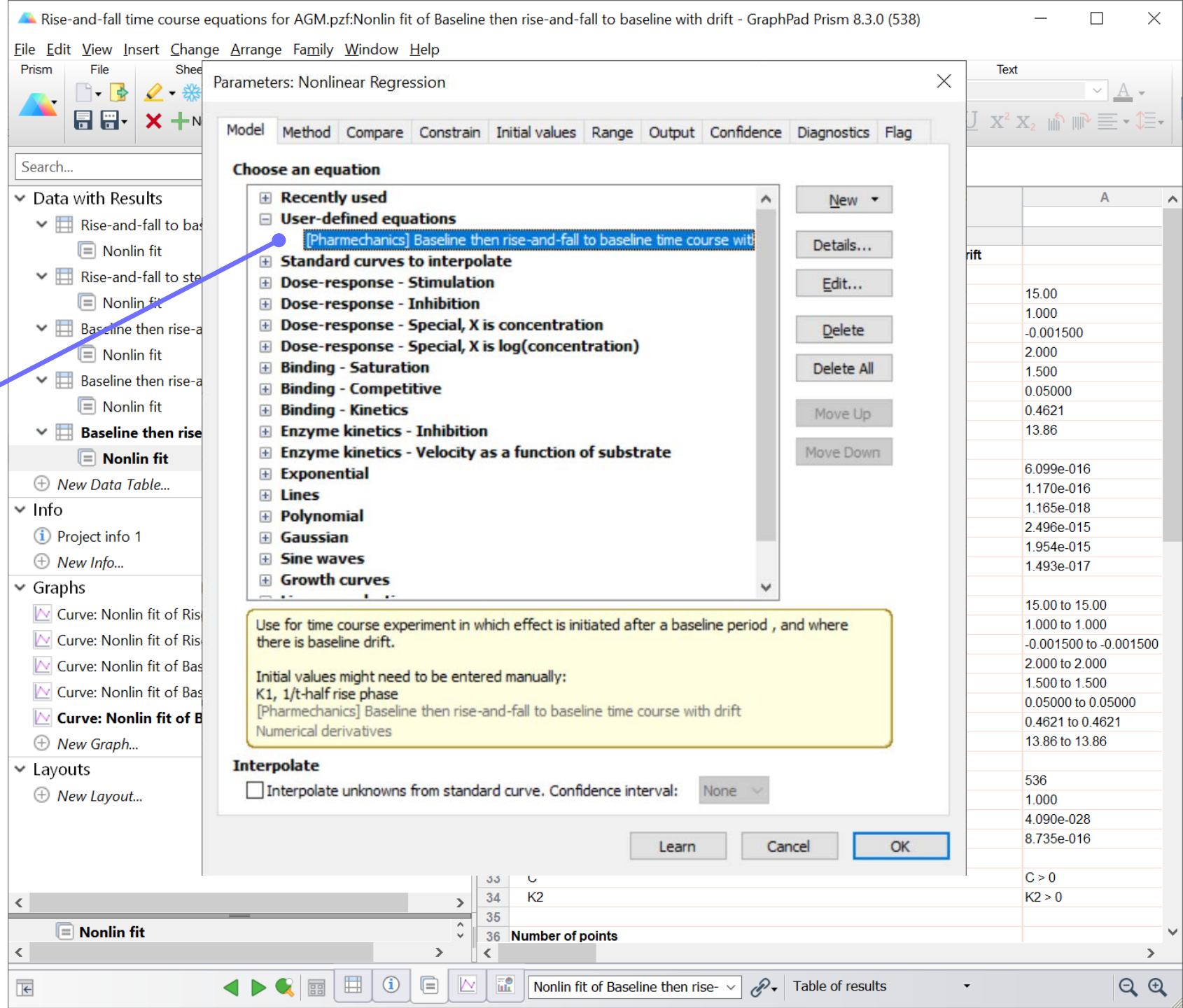


Select the equation you want by clicking on the “Nonlin fit” Results tab.

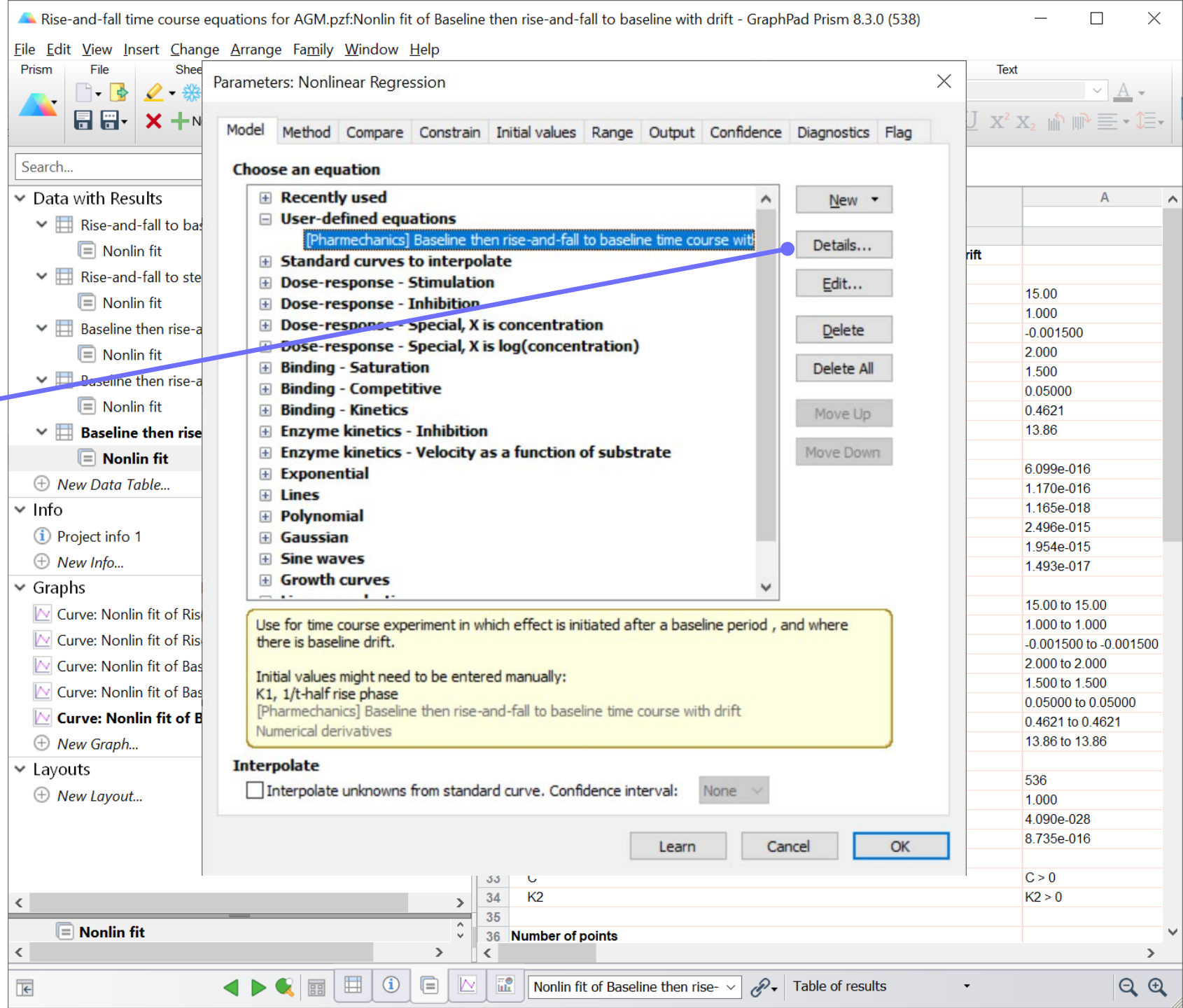


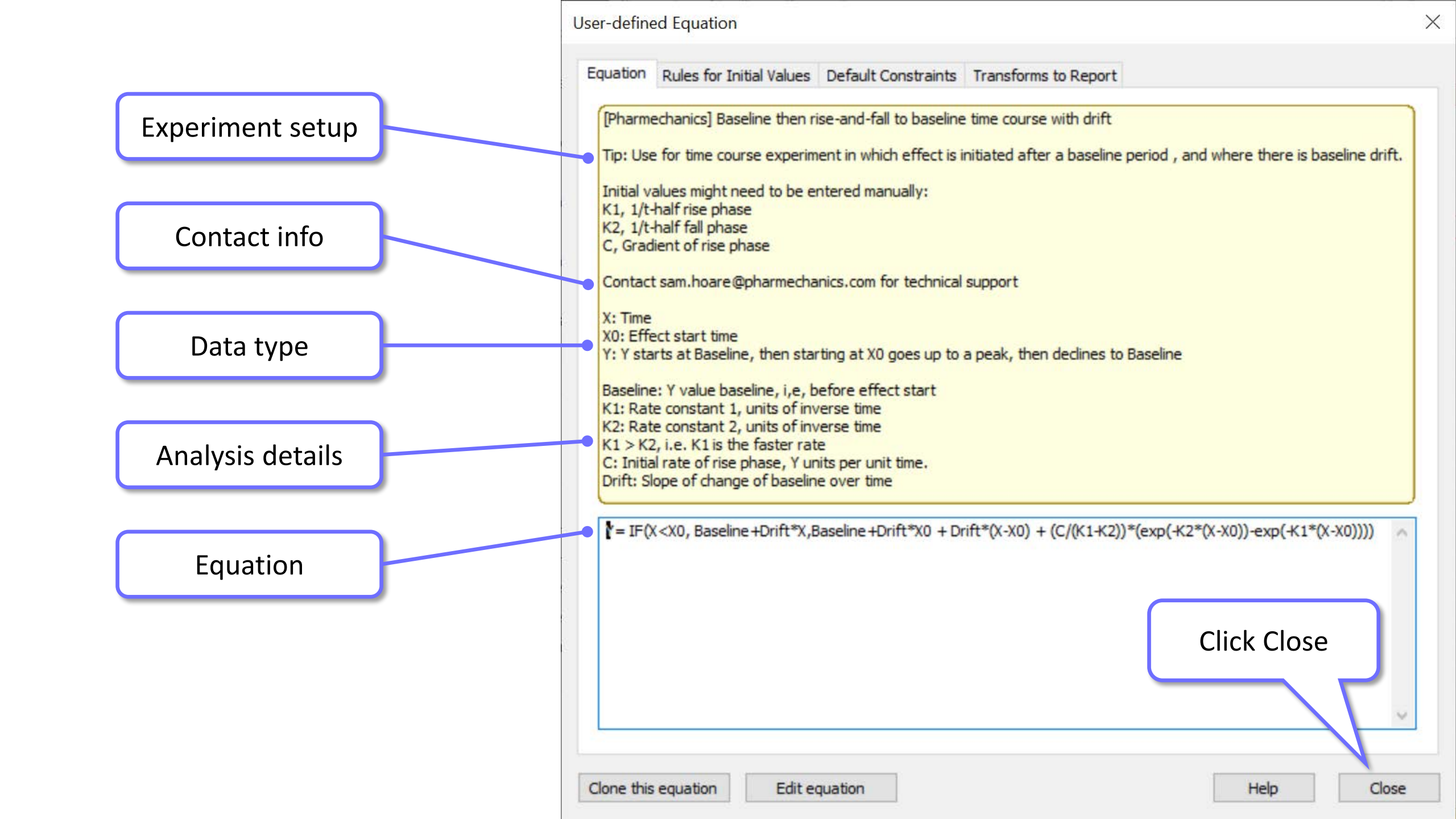
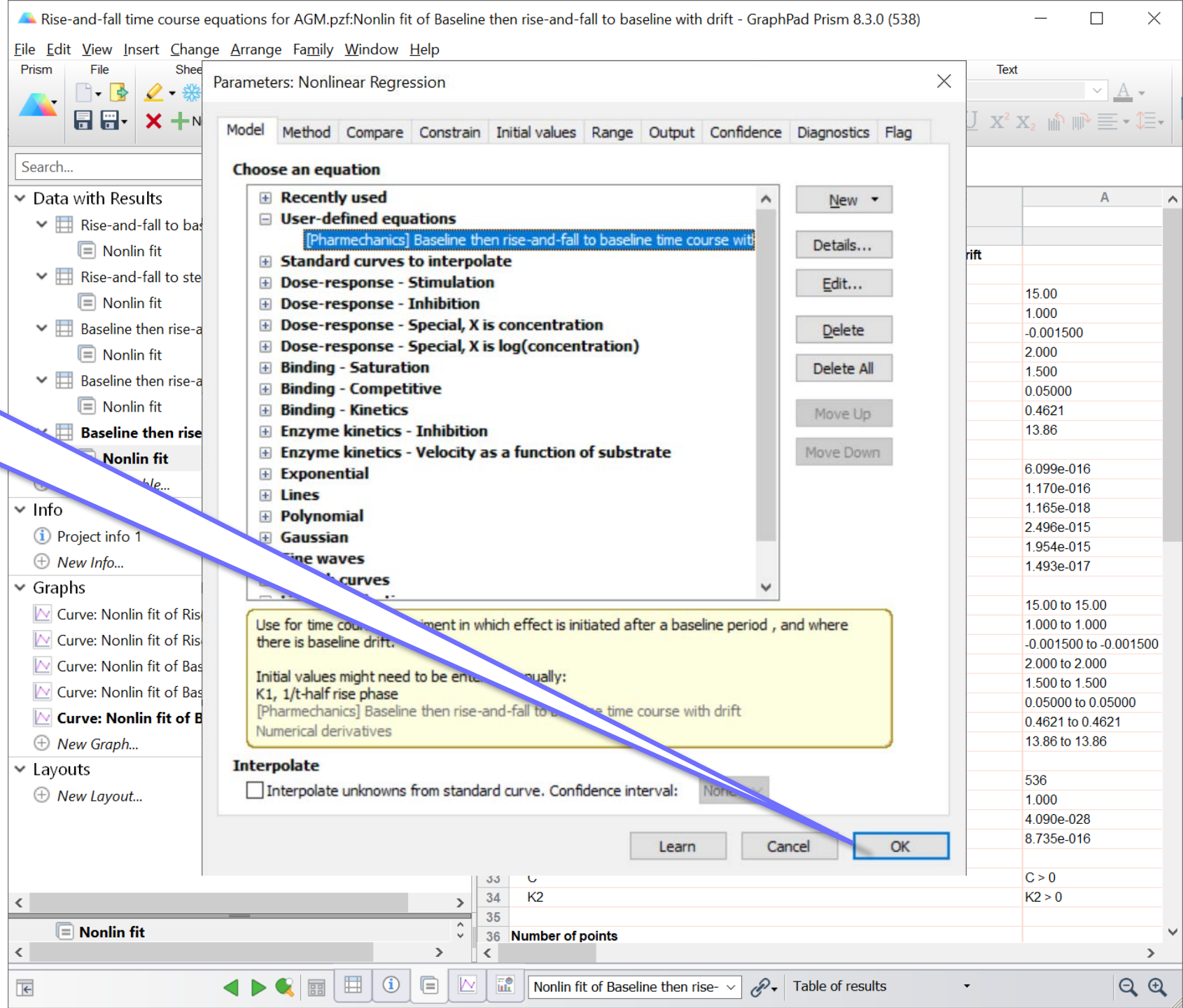

Rise-and-fall time course equations for AGM.pzf:Nonlin fit of Baseline then rise-and-fall to baseline with drift - GraphPad Prism 8.3.0 (538)
File Edit View Insert Change Arrange Family Window Help
Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text
New
Analyze
Search...
Data with 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 state
Curve: Nonlin fit of Baseline then rise-and-fall to baseline wi
New Graph...
Layouts
New Layout...
Nonlin fit
Table of results
Nonlin fit
Table of results
A
[Pharm hanics] Baseline then rise-and-fall to baseline time course with drift
Best-fit v
X0 15.00
Baseline 1.000
Drift -0.001500
C 2.000
K1 1.500
K2 0.05000
Half-time K1 0.4621
Half-time K2 13.86
Std. Error
X0 6.099e-016
Baseline 1.170e-016
Drift 1.165e-018
C 2.496e-015
K1 1.954e-015
K2 1.493e-017
95% CI (asymptotic)
X0 15.00 to 15.00
Baseline 1.000 to 1.000
Drift -0.001500 to -0.001500
C 2.000 to 2.000
K1 1.500 to 1.500
K2 0.05000 to 0.05000
Half-time K1 0.4621 to 0.4621
Half-time K2 13.86 to 13.86
Goodness of Fit
Degrees of Freedom 536
R squared 1.000
Sum of Squares 4.090e-028
Sy.x 8.735e-016
Constraints
C C > 0
K2 K2 > 0
Number of points
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 baseline time course with drift
Tip: Use for time course experiment in which effect is initiated after a baseline period , and where there is baseline drift.
Initial values might need to be entered manually:
K1, 1/t-half rise phase
K2, 1/t-half fall phase
C, Gradient of rise phase
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 Baseline
Baseline: Y value baseline, i,e, before effect start
K1: Rate constant 1, units of inverse time
K2: Rate constant 2, units of inverse time
K1 > K2, i.e. K1 is the faster rate
C: Initial rate of rise phase, Y units per unit time.
Drift: Slope of change of baseline over time
Y = IF(X<X0, Baseline+Drift*X,Baseline+Drift*X0 + Drift*(X-X0) + (C/(K1-K2))*(exp(-K2*(X-X0))-exp(-K1*(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.