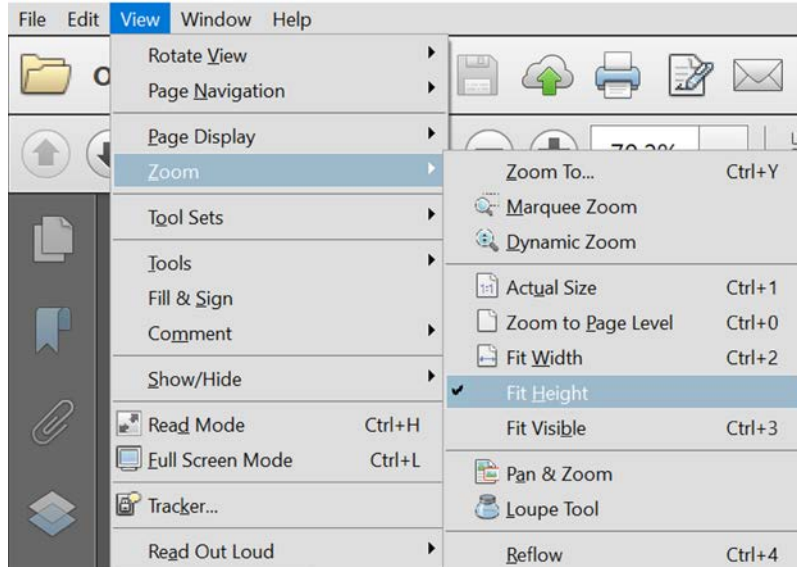


Prism 8 curve fitting to rise-and-fall to steady-state exponential equation

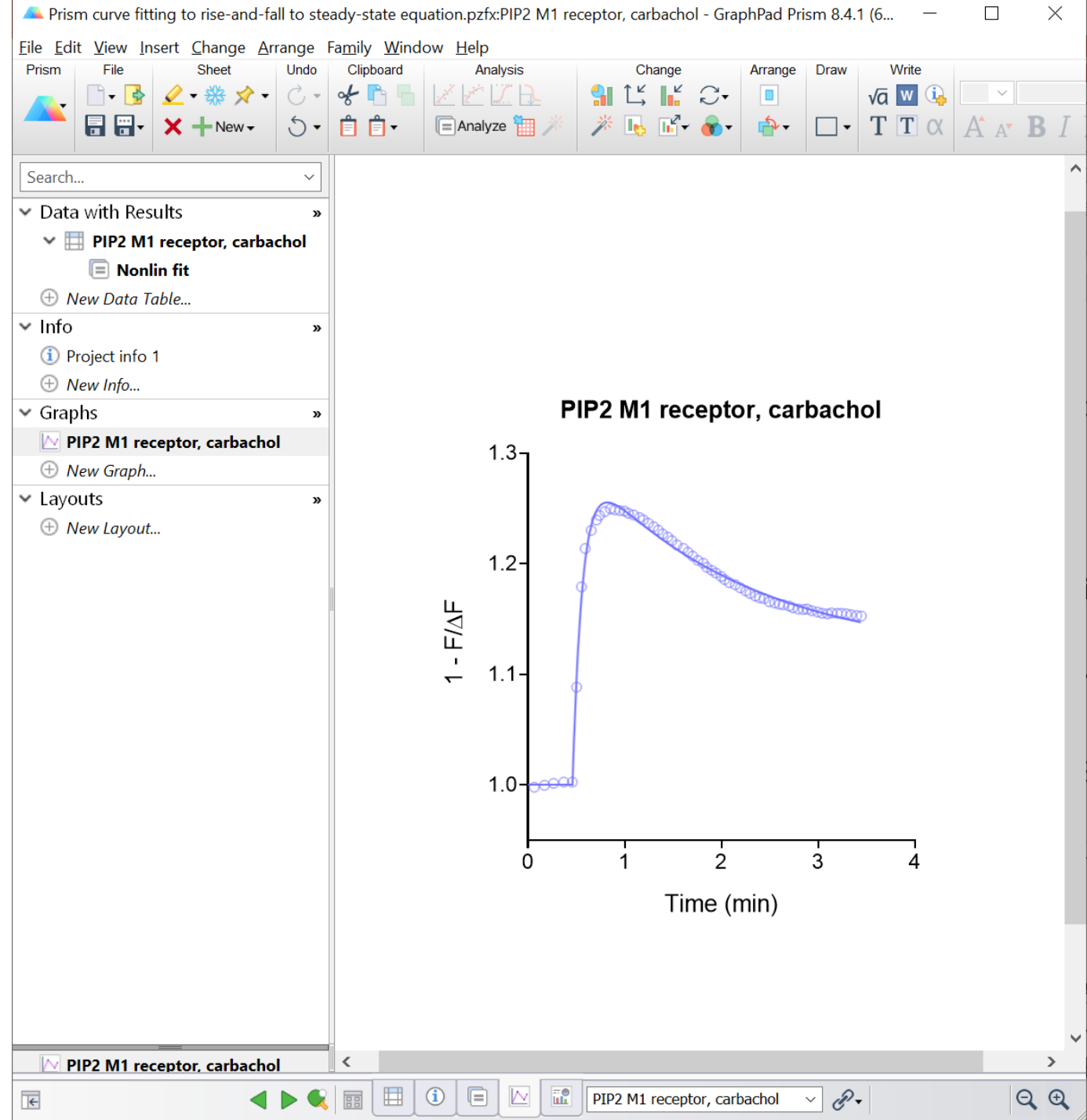


“Fit height” enables page flipping in PDF viewer

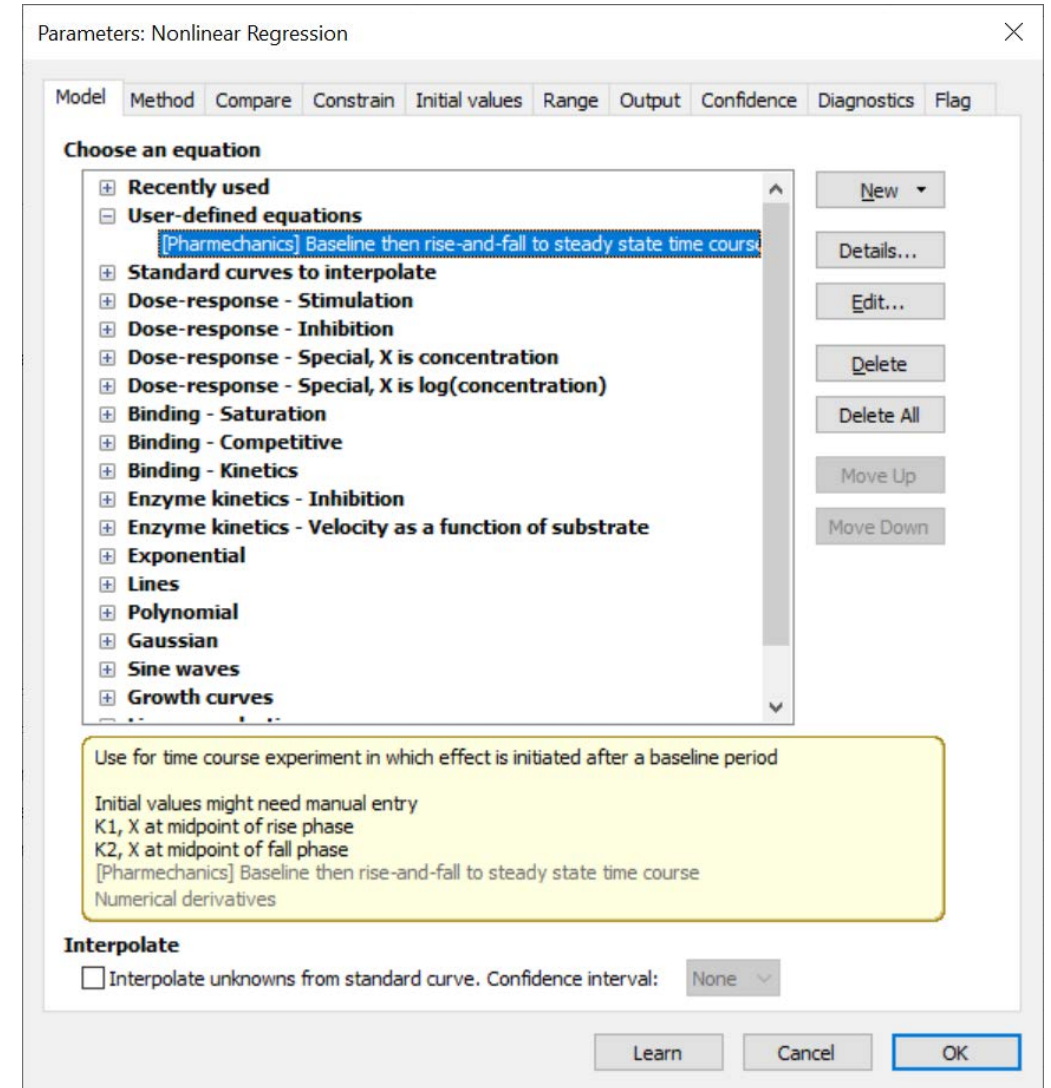
Here we are going to fit biosensor time course data to the rise-and-fall to steady-state equation.

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”



Prism curve fitting to rise-and-fall to steady-state equation.pzfx:PIP2 M1 receptor, carbachol - GraphPad Prism 8.4.1 (676)

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Change Import Draw Write

Search... Table format: XY

▼ Data with Results »

- PIP2 M1 receptor, carbachol
- + New Data Table...

▼ Info »

- Project info 1
- + New Info...

▼ Graphs »

- PIP2 M1 receptor, carbachol
- + New Graph...

▼ Layouts »

- + New Layout...

	X	Group A	Group B	Group C	Group D	Group E
	Time (min)	Carbachol	Title	Title	Title	Title
	X	Y	Y	Y	Y	Y
1	Title	-0.022	0.997			
2	Title	0.066	0.998			
3	Title	0.172	0.999			
4	Title	0.266	1.001			
5	Title	0.371	1.002			
6	Title	0.460	1.002			
7	Title	0.504	1.088			
8	Title	0.554	1.179			
9	Title	0.592	1.214			
10	Title	0.653	1.230			
11	Title	0.709	1.239			
12	Title	0.740	1.244			
13	Title	0.795	1.247			
14	Title	0.855	1.249			
15	Title	0.899	1.249			
16	Title	0.951	1.248			
17	Title	0.999	1.247			
18	Title	1.039	1.245			
19	Title	1.095	1.244			
20	Title	1.154	1.242			
21	Title	1.194	1.240			
22	Title	1.246	1.236			
23	Title	1.301	1.233			
24	Title	1.349	1.230			
25	Title	1.394	1.227			
26	Title	1.445	1.224			
27	Title	1.489	1.221			
28	Title	1.541	1.217			
29	Title	1.608	1.214			
30	Title	1.656	1.210			
31	Title	1.704	1.207			
32	Title	1.759	1.203			
33	Title	1.814	1.200			

PIP2 M1 receptor, carbachol

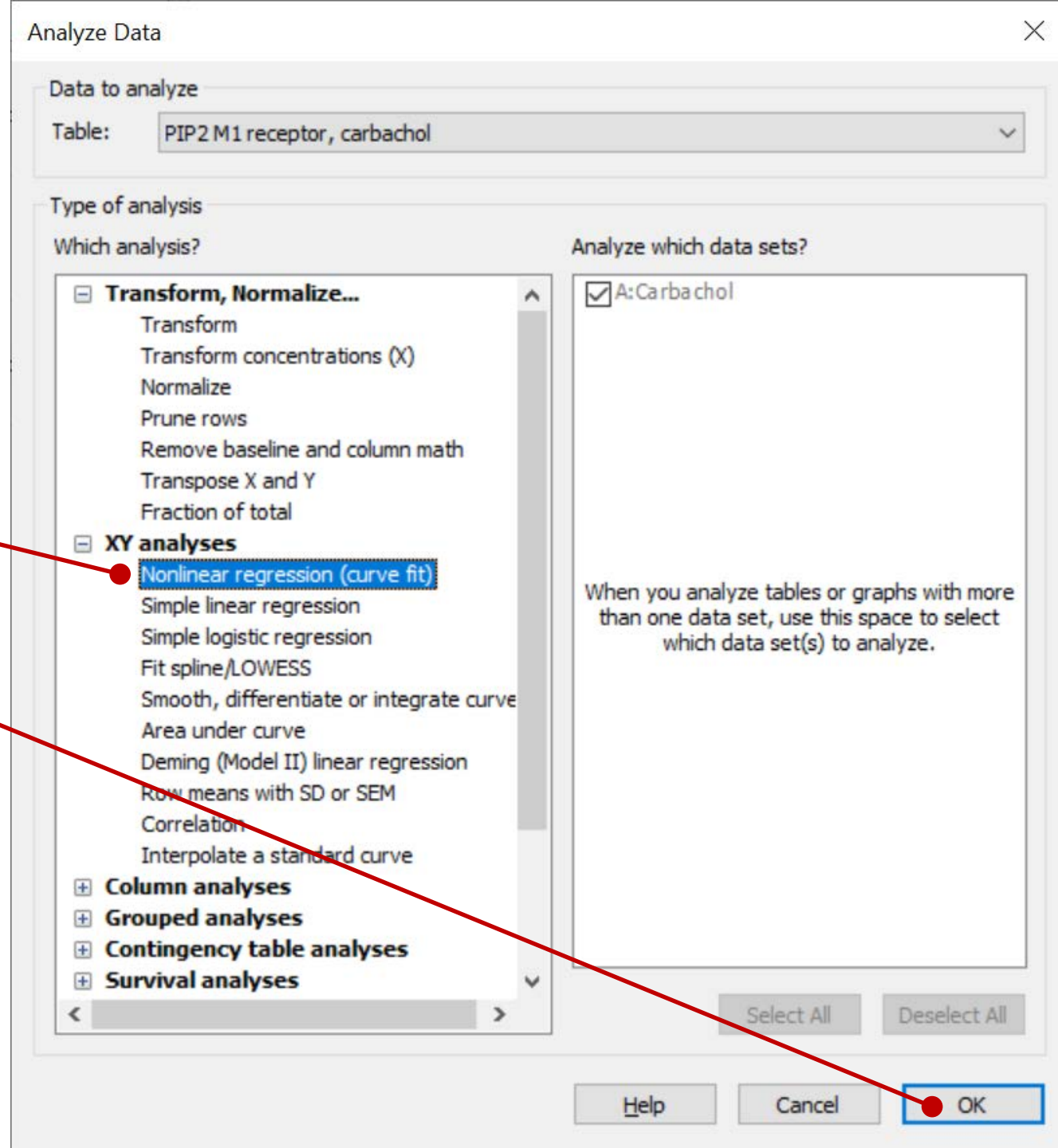
Row 59, Column

Enter data into Prism data table

This brings up the “Analyze Data”
dialogue

Select “Nonlinear regression”

Click “OK”

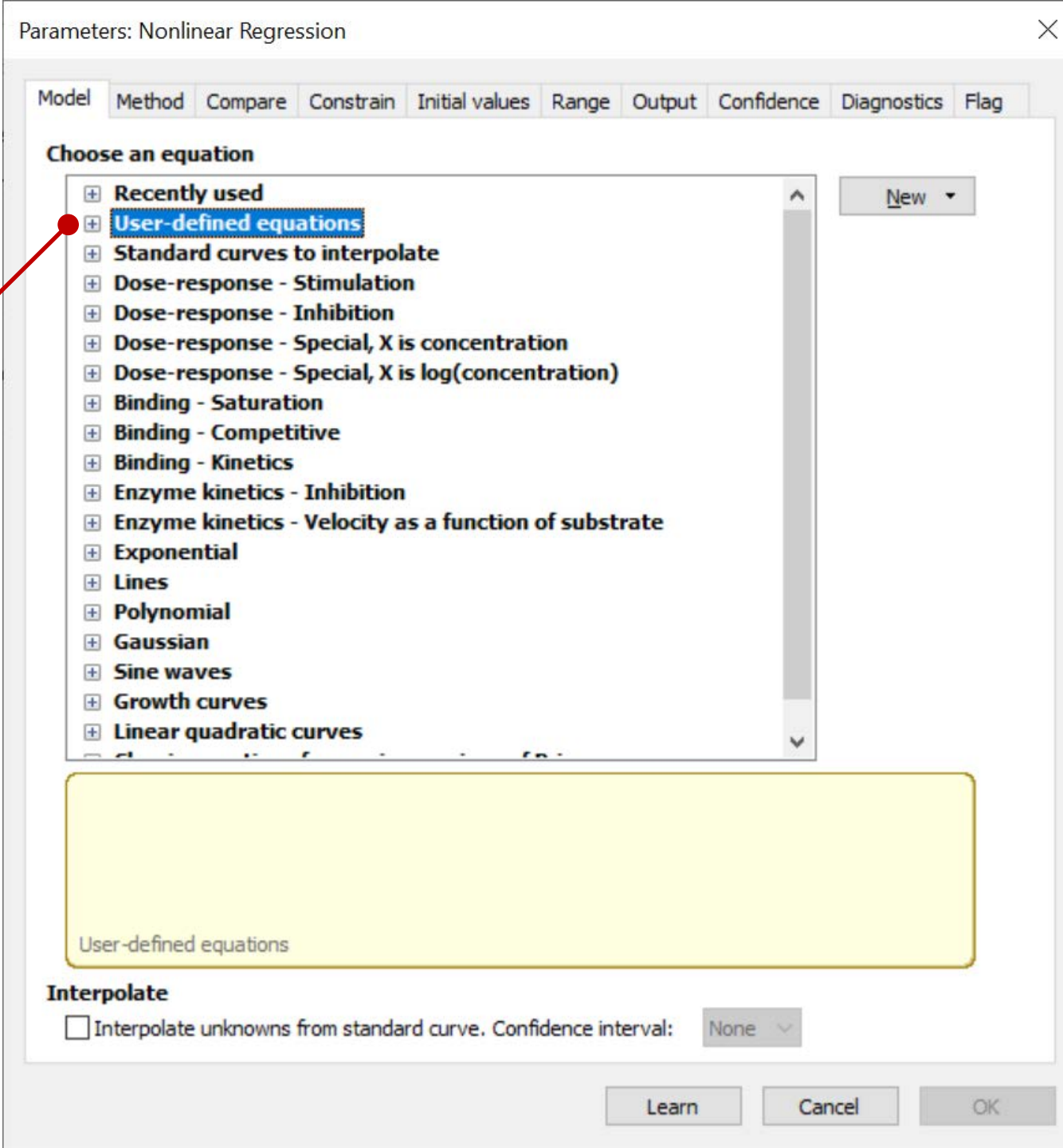


This brings up the “Parameters: Nonlinear Regression” dialogue.

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

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Choose an equation

- Recently used
- User-defined equations
 - [Pharmechanics] Baseline then rise-and-fall to steady state time course
- Standard curves to interpolate
- Dose-response - Stimulation
- Dose-response - Inhibition
- Dose-response - Special, X is concentration
- Dose-response - Special, X is log(concentration)
- Binding - Saturation
- Binding - Competitive
- Binding - Kinetics
- Enzyme kinetics - Inhibition
- Enzyme kinetics - Velocity as a function of substrate
- Exponential
- Lines
- Polynomial
- Gaussian
- Sine waves
- Growth curves

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

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

Numerical derivatives

Interpolate

Interpolate unknowns from standard curve. Confidence interval: None

Learn Cancel OK

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)

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Select Data Set Select All

PIP2 M1 receptor, carbachol:A:Carbachol

To select several data sets, press Control or Shift while selecting.

Parameter Name	Choose Automatically	Initial Value	Hook
X0	<input checked="" type="checkbox"/>	0.3449612	
Baseline	<input checked="" type="checkbox"/>	0.997059	
SteadyState	<input checked="" type="checkbox"/>	0.252353	
D	<input checked="" type="checkbox"/>	0.218061894795	
K1	<input checked="" type="checkbox"/>	5.835221513761	
K2	<input checked="" type="checkbox"/>	0.175056645412	

Learn Cancel OK

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

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Select Data Set Select All

PIP2 M1 receptor, carbachol:A:Carbachol

To select several data sets, press Control or Shift while selecting.

Parameter Name	Choose Automatically	Initial Value	Hook
X0	<input type="checkbox"/>	0.45	
Baseline	<input checked="" type="checkbox"/>	0.997059	
SteadyState	<input type="checkbox"/>	0.18	
D	<input checked="" type="checkbox"/>	0.218061894795	
K1	<input checked="" type="checkbox"/>	5.835221513761	
K2	<input checked="" type="checkbox"/>	0.175056645412	

Learn Cancel OK

“Asymmetrical” is the rigorous error calculation method

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

Parameters: Nonlinear Regression

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Confidence intervals (CI) of parameters

Calculate CI of parameters

Confidence level: 95%

Output Format: Range ("1.23 to 4.56")

Asymmetrical (profile-likelihood) CI

Recommended because they are more accurate. Can be slow.

Compute even when the fit is ambiguous and the CIs would be difficult to interpret.

Symmetrical (asymptotic) approximate CI

Less accurate so not recommended. Matches Prism 1-6 and most programs. Faster to calculate.

Show SE of parameters

Confidence or prediction bands

Plot confidence/prediction bands

Confidence level: 95%

Confidence bands

Confidence bands show you the likely location of the TRUE curve.

Prediction bands

Prediction bands show you the likely location of additional data points.

Ambiguous fits and unstable parameters

Identify “ambiguous” fits. Matches Prism 8.1 and earlier.

Identify “unstable” parameters. A new (8.2) feature from Prism Labs.

Neither. Just show the best-fit values even when the fit is problematic.

Make these choices the default for future fits.

Learn

Cancel

OK

Model Method Compare Constrain Initial values Range Output Confidence Diagnostics Flag

Confidence intervals (CI) of parameters

Calculate CI of parameters

Confidence level: 95% ▾

Output Format: Range ("1.23 to 4.56") ▾

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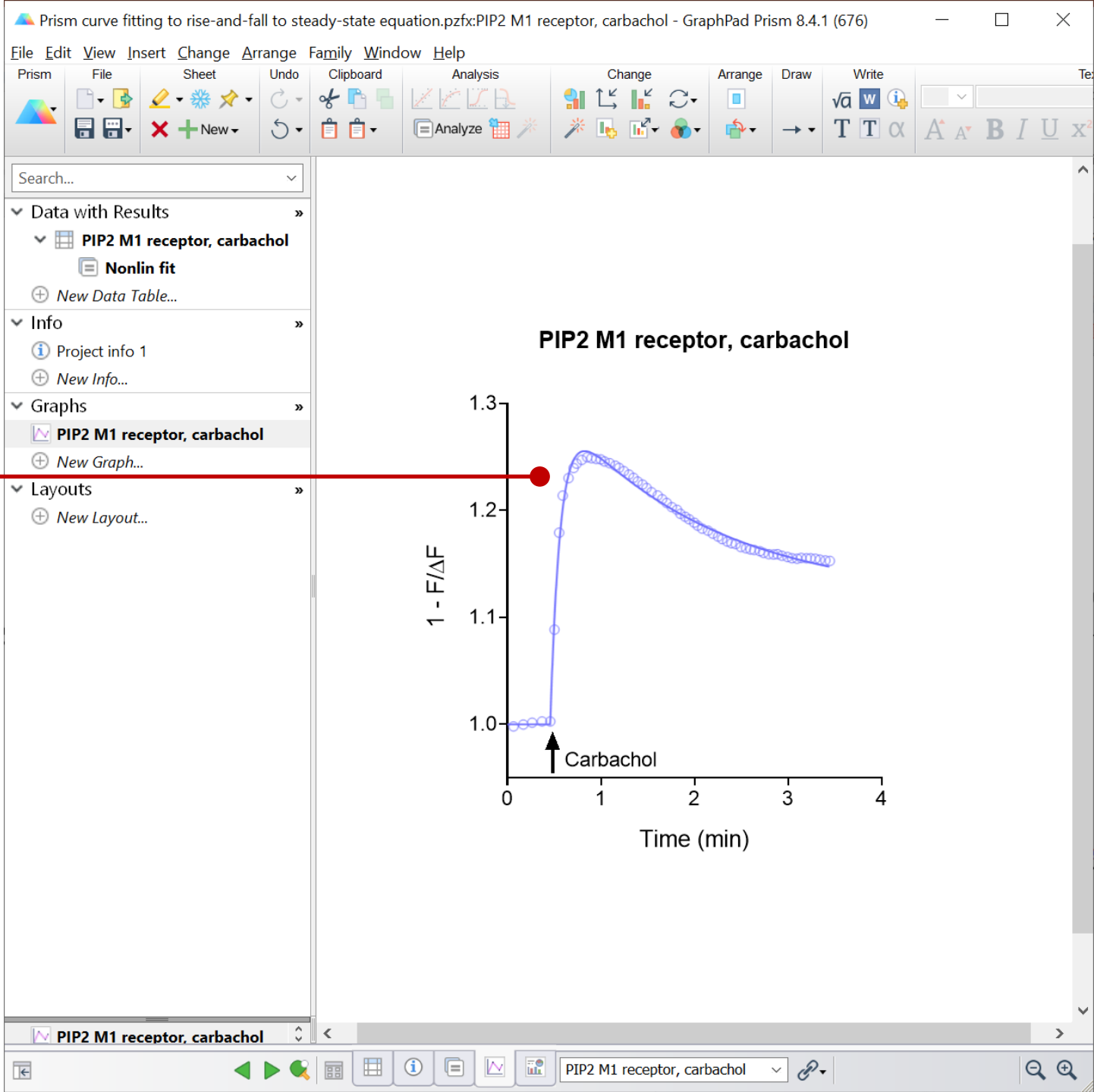
Make these choices the default for future fits.

Learn

Cancel

OK

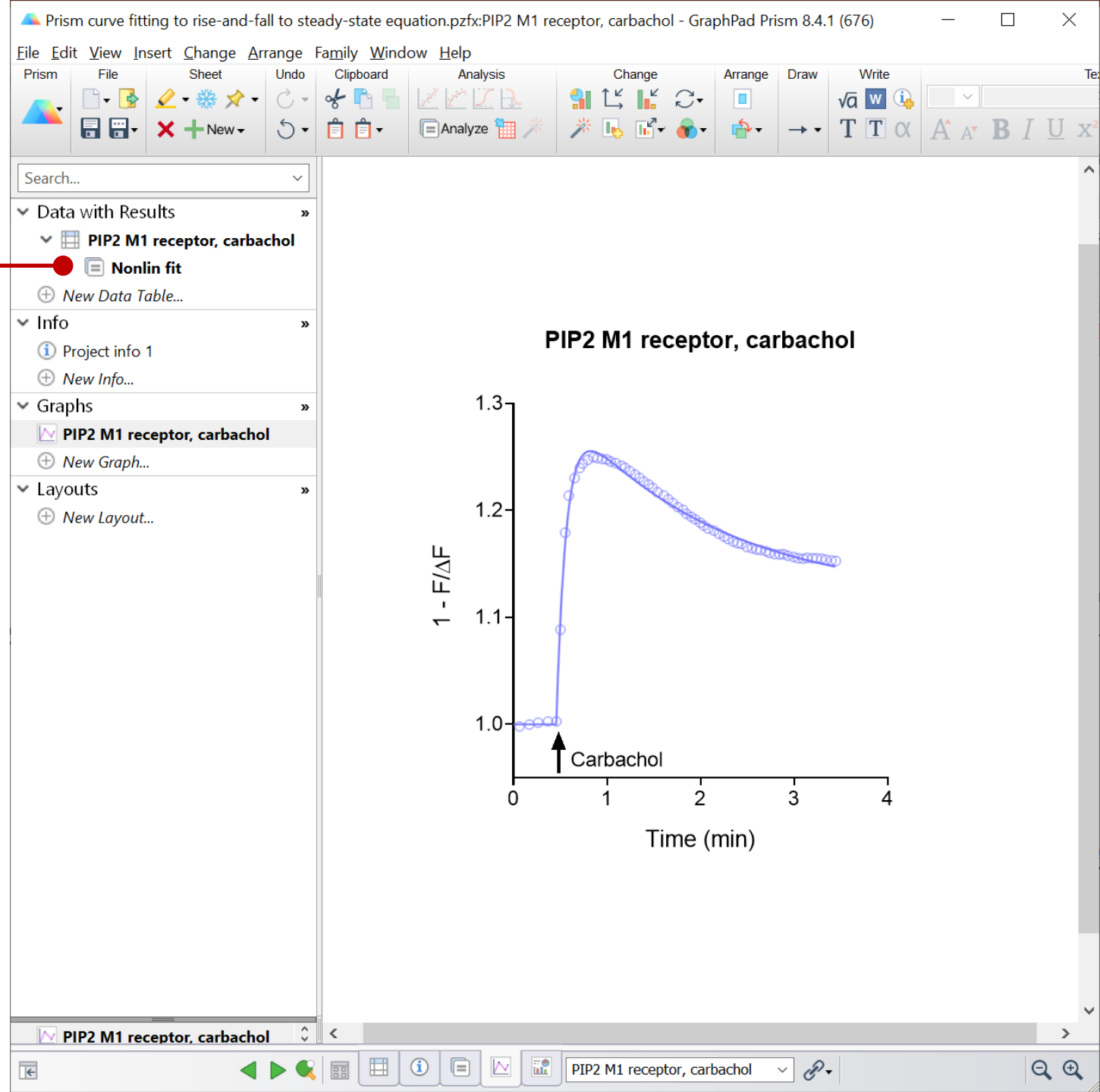
Click "OK" to run the analysis



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: $R^2 = 0.995$

Nonlin fit		A	B
Table of results		Carbachol	
1	[Pharmacokinetics] Baseline then rise-and-fall to steady state time course		
2	Best-fit values		
3	X0	0.4589	
4	Baseline	0.9995	
5	SteadyState	0.1169	
6	D	2.585	
7	K1	9.390	
8	K2	0.6032	
9	Half-time K1	0.07382	
10	Half-time K2	1.149	
11	Std. Error		
12	X0	0.001782	
13	Baseline	0.002089	
14	SteadyState	0.007750	
15	D	0.1334	
16	K1	0.3905	
17	K2	0.06261	
18	95% CI (asymptotic)		
19	X0	0.4553 to 0.4624	
20	Baseline	0.9954 to 1.004	
21	SteadyState	0.1014 to 0.1324	
22	D	2.318 to 2.852	
23	K1	8.608 to 10.17	
24	K2	0.4780 to 0.7285	
25	Half-time K1	0.06815 to 0.08052	
26	Half-time K2	0.9515 to 1.450	
27	Goodness of Fit		
28	Degrees of Freedom	60	
29	R squared	0.9954	
30	Sum of Squares	0.001309	
31	Sy.x	0.004671	
32	Constraints		
33	SteadyState	SteadyState > 0	
34	D	D > 0	
35	K1	K1 > 1*K2	
36	K2	K2 > 0	

Calculating the initial rate and k_{τ}

Use this formula to calculate the initial rate:

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

$$\text{Initial rate} = 2.73 \text{ NFU} \cdot \text{min}^{-1}$$

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

Prism curve fitting to rise-and-fall to steady-state equation.pzfx:Nonlin fit of PIP2 M1 receptor, carbachol - GraphPad Prism 8...

File Edit View Insert Change Arrange Family Window Help

Prism File Sheet Undo Clipboard Analysis Interpret Change Draw Write Text

Search...

Table of results

Nonlin fit		A	B
Table of results		Carbachol	
1	[Pharmacokinetics] Baseline then rise-and-fall to steady state time course		
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17	K2	0.06261	
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31	Sy.x	0.004671	
32	Constraints		
33	SteadyState	SteadyState > 0	
34	D	D > 0	
35	K1	K1 > 1*K2	
36	K2	K2 > 0	

Nonlin fit

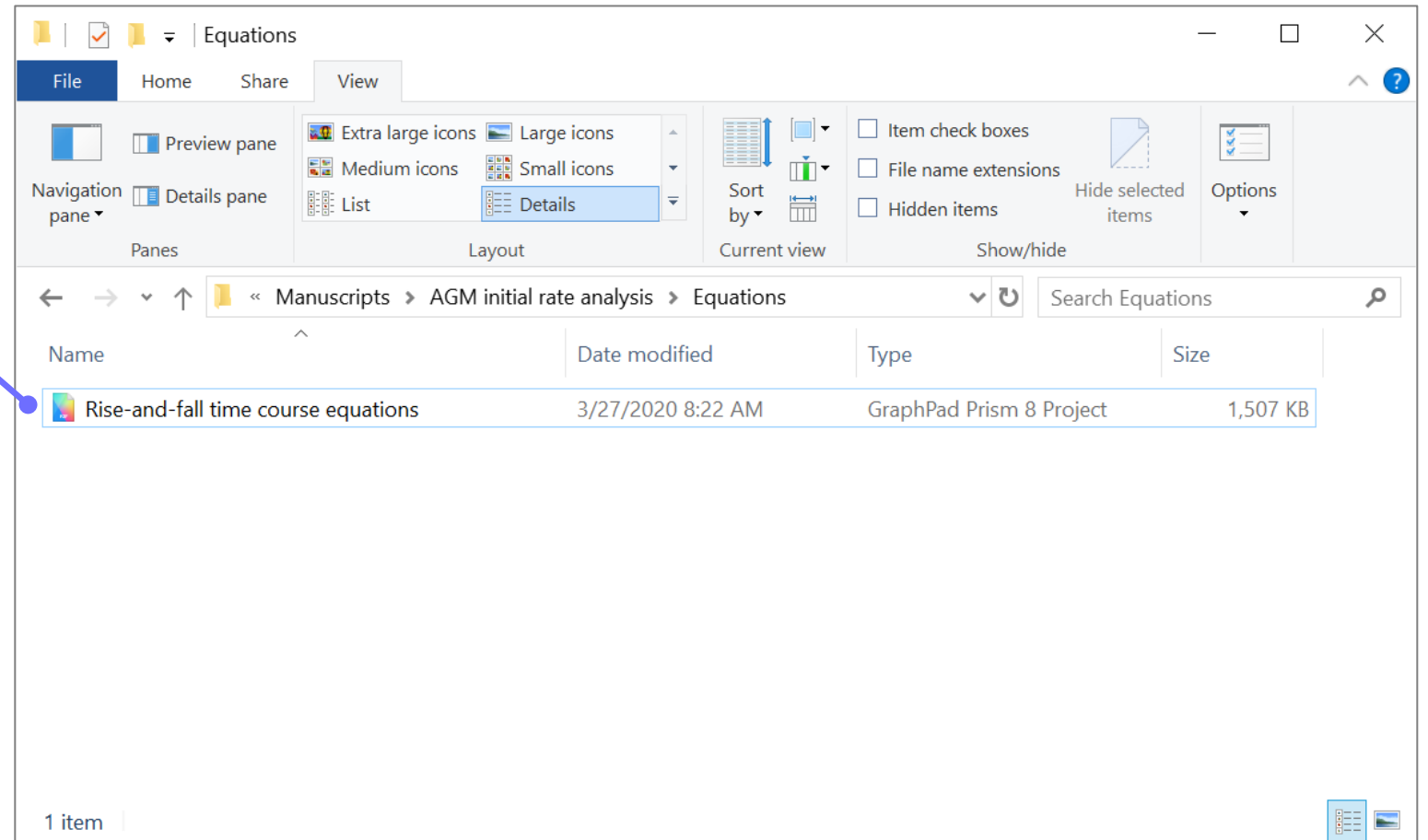
Nonlin fit of PIP2 M1 receptor, ... Table of results

NFU, normalized fluorescence units

Loading equations into Prism from a file

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

Then open it.

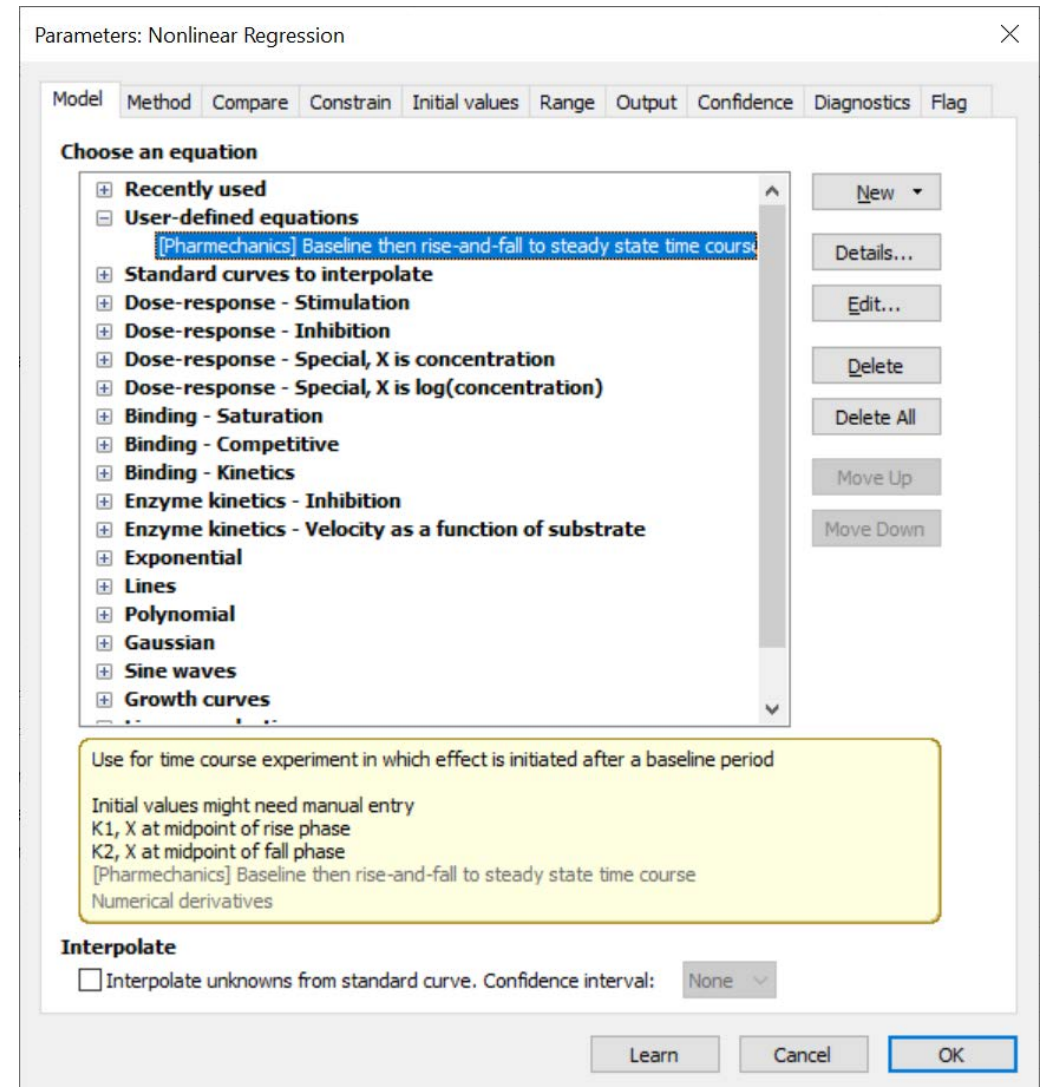


GraphPad Prism contains an equation editor for the input of user-defined equations.

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.



Rise-and-fall time course equations for AGM.pzf:Curve: 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 Change Arrange Draw Write Text

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 with dri
 - ⊕ New Graph...
- ▼ Layouts
 - ⊕ New Layout...

Curve: Nonlin fit of Baseline then rise-and-fall to steady : ^

Y value

Time

X0

SteadyState

Baseline

Curve: Nonlin fit of Baseline then rise-and-fall to steady

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"

Rise-and-fall time course equations for AGM.pzf:Curve: 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 Change Arrange Draw Write Text

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 with dri
 - + New Graph...
- ▼ Layouts
 - + New Layout...

Y value

SteadyState

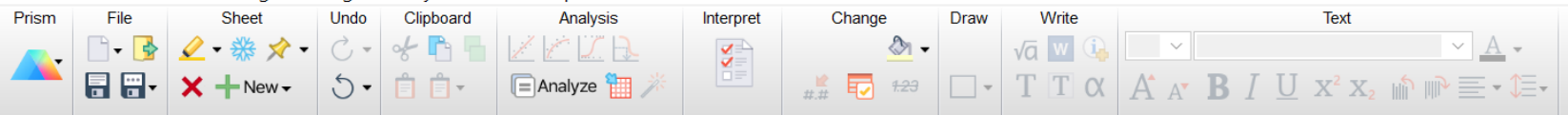
Baseline

X0

Time

Curve: Nonlin fit of Baseline then rise-and-fall to steady state

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



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 with drift
 - ⊕ New Graph...
- ▼ Layouts
 - ⊕ New Layout...

Nonlin fit

Table of results

Nonlin fit		A	B
Table of results			
1	[Pharmacokinetics] Baseline then rise-and-fall to steady state time course		
2	Best-fit values		
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			
37			

Click this icon

Equation Rules for Initial Values Default Constraints Transforms to Report

Experiment setup

Contact info

Data type

Analysis details

Equation

[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

$$Y = \text{IF}(X < X0, \text{Baseline}, \text{Baseline} + \text{SteadyState} * (1 - D * \exp(-K1 * (X - X0)) + (D - 1) * \exp(-K2 * (X - X0))))$$

Click Close

Clone this equation

Edit equation

Help

Close

