Curve Fitting

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Outline

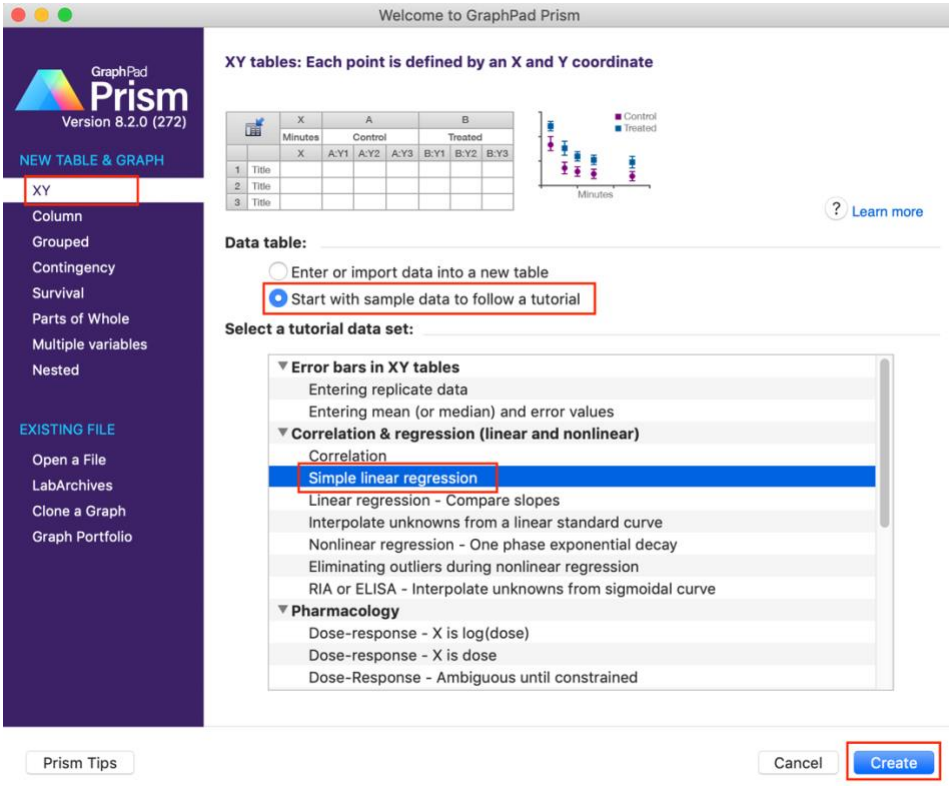
1. How to perform curve fitting?
2. Comparing models/curves
3. How to customize equation of models?

1. How to perform curve fitting

1) Simple Linear Regression

Let's take simple linear regression with curve fitting as an example. Linear regression fits a straight line through your data to find the best-fit value of the slope and intercept.

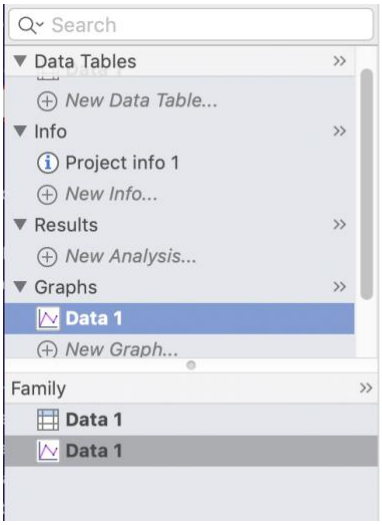
Choose the **sample data** with XY data table: **Simple Linear Regression**

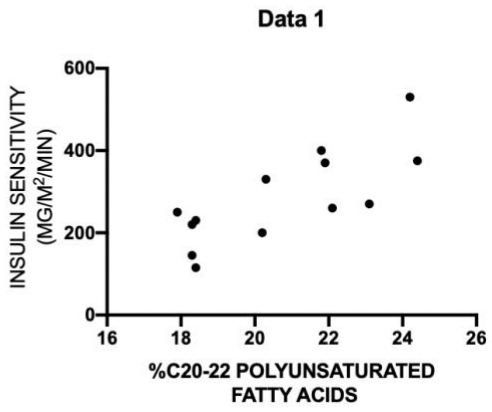


In this example, the X values are the percentage of a certain kind of fatty acid in muscle (from a biopsy) and the Y values are insulin sensitivity.

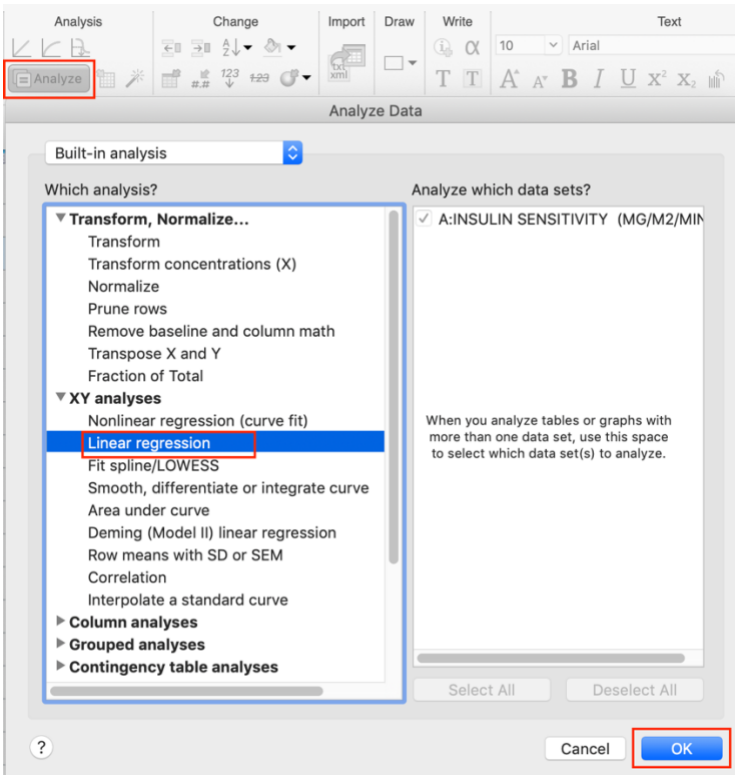
		X	Group A
		%C20-22 POLYUNSATURATED FATTY ACIDS	INSULIN SENSITIVITY (MG/M^2/MIN)
		X	Y
1	Titl	17.9	250
2	Titl	18.3	220
3	Titl	18.3	145
4	Titl	18.4	115
5	Titl	18.4	230
6	Titl	20.2	200
7	Titl	20.3	330
8	Titl	21.8	400
9	Titl	21.9	370
10	Titl	22.1	260
11	Titl	23.1	270
12	Titl	24.2	530
13	Titl	24.4	375

The corresponding graph is like:

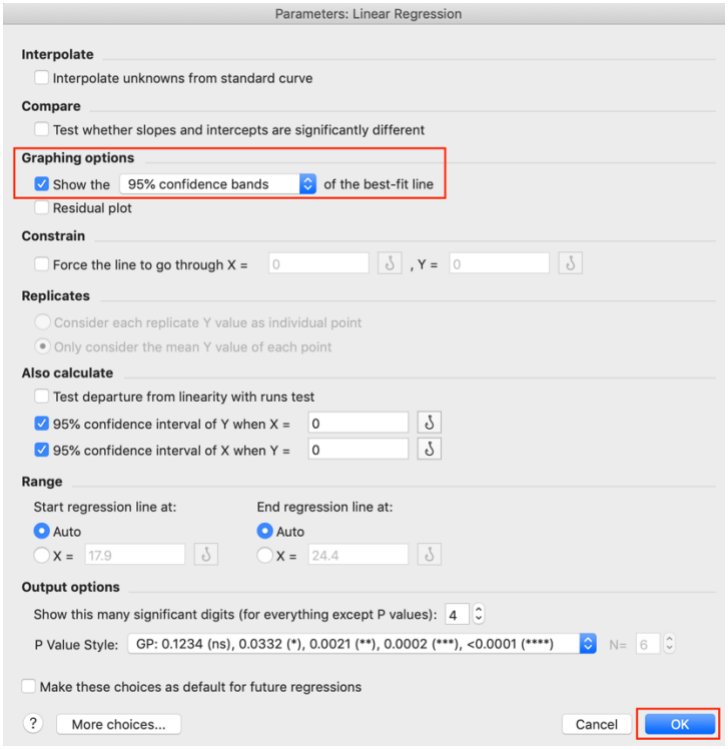




Click "Analyze". In the Analyze Data dialog, **select "Linear regression"** under the "XY analyses". Then **click "OK"**.



In the "Parameters: Linear Regression" dialog, check the **"Show the 95% confidence bands of the best-fit line"** then **click "OK"**.



Then Prism presents a full set of linear regression results.

Tabular results

	Linear reg. Tabular results	A INSULIN SENSITIVITY (MG/M^2/MIN)
Part 1		Y
1	**Best-fit values**	
2	Slope	37.21
3	Y-intercept	-486.5
4	X-intercept	13.08
5	1/slope	0.02688
6		
7	**Std. Error**	
8	Slope	9.296
9	Y-intercept	193.7
10		
11	**95% Confidence Intervals**	
12	Slope	16.75 to 57.67
13	Y-intercept	-912.9 to -60.18
14	X-intercept	3.562 to 15.97
15 Part 2		
16	**Goodness of Fit**	
17	R square	0.5929
18	Sy.x	75.90
19		
20	**Is slope significantly non-zero?**	
21	F	16.02
22	DFn, DFd	1, 11
23	P value	0.0021
24	Deviation from zero?	Significant
25 Part 1		
26	**Equation**	Y = 37.21*X - 486.5

Results Explanation:

a) Part 1: Slope and Intercept

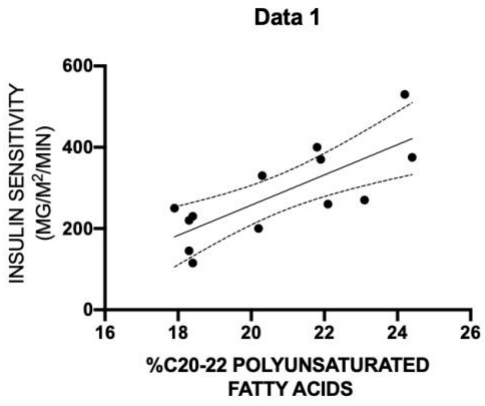
Prism first reports the best-fit values of the slope and intercept. It also reports the X intercept and the reciprocal of the slope.

At the bottom of the results page, the slope and intercept are reported again in the form of the equation that defines the best-fit line. You can copy this equation and paste onto a graph, or into a manuscript. In this case, the best-fit line is Y = 37.21*X – 486.5

b) Part 2: Goodness-of-fit of linear regression

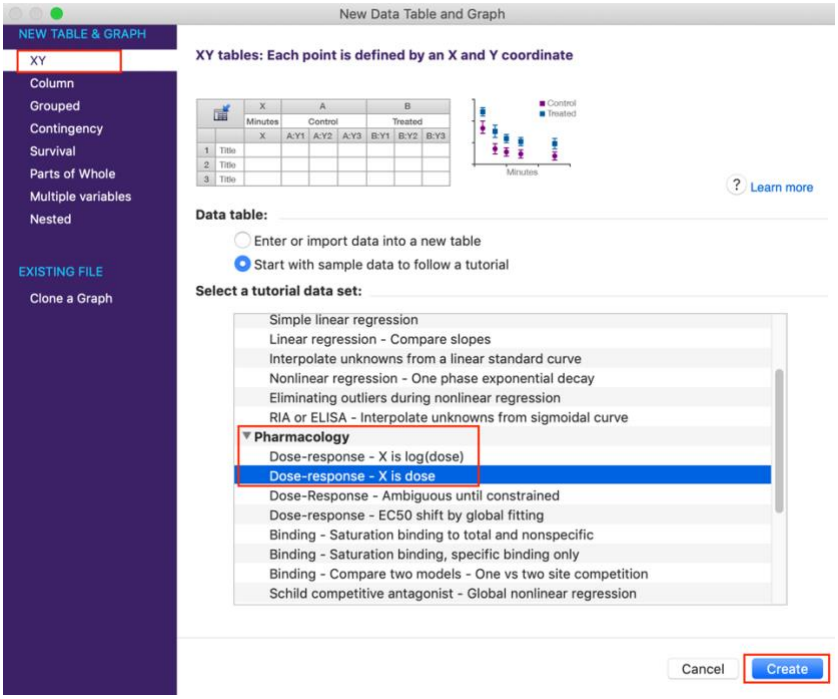
Prism provides R-square for goodness-of-fit. You can think of R-square as the fraction of the total variance of Y that is “explained” by variation in X.

In the corresponding graph, two confidence bands surrounding the best-fit line define the confidence interval of the best-fit line. The dashed confidence bands are curved and they are the boundaries of all possible straight lines.



2) Nonlinear Regression: Dose-response – X is dose

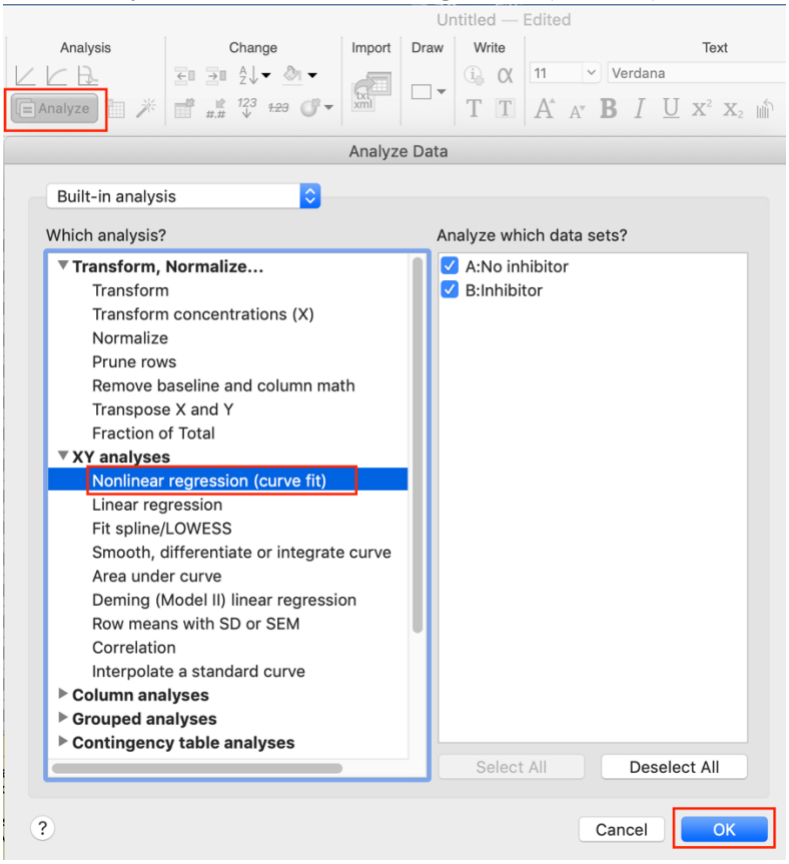
Then let's try a nonlinear regression example. Choose the **sample data** with XY data table: **Does-response – X is dose.**



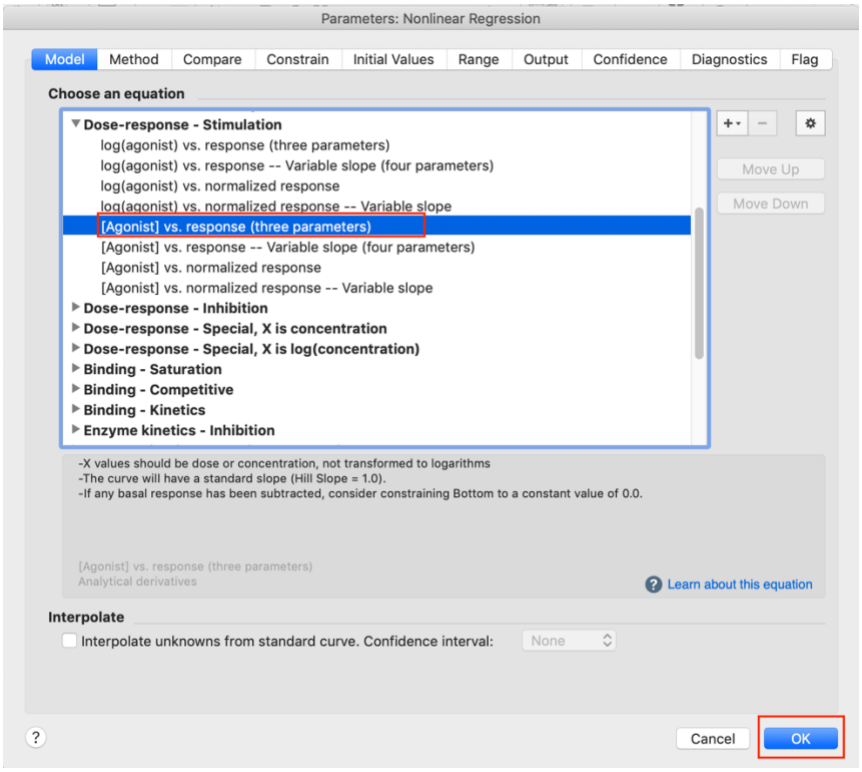
In this example, the X values are molar concentration. The Y values are responses (in triplicate) in two experimental conditions. We would like to fit a dose-response curve to determine the EC50 of the drug, and its Hill Slope, in both conditions.

		X	Group A			Group B		
		[Agonist], M	No inhibitor			Inhibitor		
		X	A:Y1	A:Y2	A:Y3	B:Y1	B:Y2	B:Y3
1	Title	0.000000	0	3	2	3	5	4
2	Title	1.000000e-008	11	33	25			
3	Title	3.000000e-008	125	141	160	11	25	28
4	Title	1.000000e-007	190	218	196	52	55	61
5	Title	3.000000e-007	258	289	345	80	77	44
6	Title	0.000001	322	353	328	171	195	246
7	Title	0.000003	354	359	369	289	230	243
8	Title	0.000010	348	298	372	272	333	310
9	Title	0.000030				359	306	297
10	Title	0.000100	412	378	399	352	320	365
11	Title	0.000300				389	338	

Click Analyze. Choose "**Nonlinear regression (curve fit)**" then **click "OK"**.



In the "Parameters: Nonlinear Regression" dialog, choose **"[Agonist] vs. response (three parameters)"**. **Click "OK"**.



Then the results will be like:

Table of results

	Nonlin fit Table of results	A No inhibitor	B Inhibitor
		Y	Y
1	**[Agonist] vs. response (three parameters)**		
2	**Best-fit values**		
3	Bottom	-2.228	9.125
4	Top	365.8	343.0
5	EC50	6.814e-008	9.369e-007
6	logEC50	-7.167	-6.028
7	Span	368.0	333.8
8	**95% CI (profile likelihood)**		
9	Bottom	-31.50 to 25.94	-11.72 to 29.34
10	Top	347.9 to 384.1	324.9 to 361.5
11	EC50	4.705e-008 to 9.987e-008	6.537e-007 to 1.349e-006
12	logEC50	-7.327 to -7.001	-6.185 to -5.870
13	**Goodness of Fit**		
14	Degrees of Freedom	24	26
15	R squared	0.9640	0.9660
16	Sum of Squares	19278	18248
17	Sy.x	28.34	26.49
18	**Constraints**		
19	EC50	EC50 > 0	EC50 > 0
20			
21	**Number of points**		
22	# of X values	30	33
23	# Y values analyzed	27	29

More detailed interpretation of these results could be found at: [Interpreting results: Nonlinear regression.]

2. **Comparing models**

Previously the dose-response model was generated WITHOUT a variable slope parameter. Now let's compare it with another dose-response model WITH a variable slope parameter.

Double click the header of the previous results.

Table of results

	Nonlin fit Table of results	A No inhibitor	B Inhibitor
		Y	Y
1	**[Agonist] vs. response (three parameters)**		
2	**Best-fit values**		
3	Bottom	-2.228	9.125
4	Top	365.8	343.0
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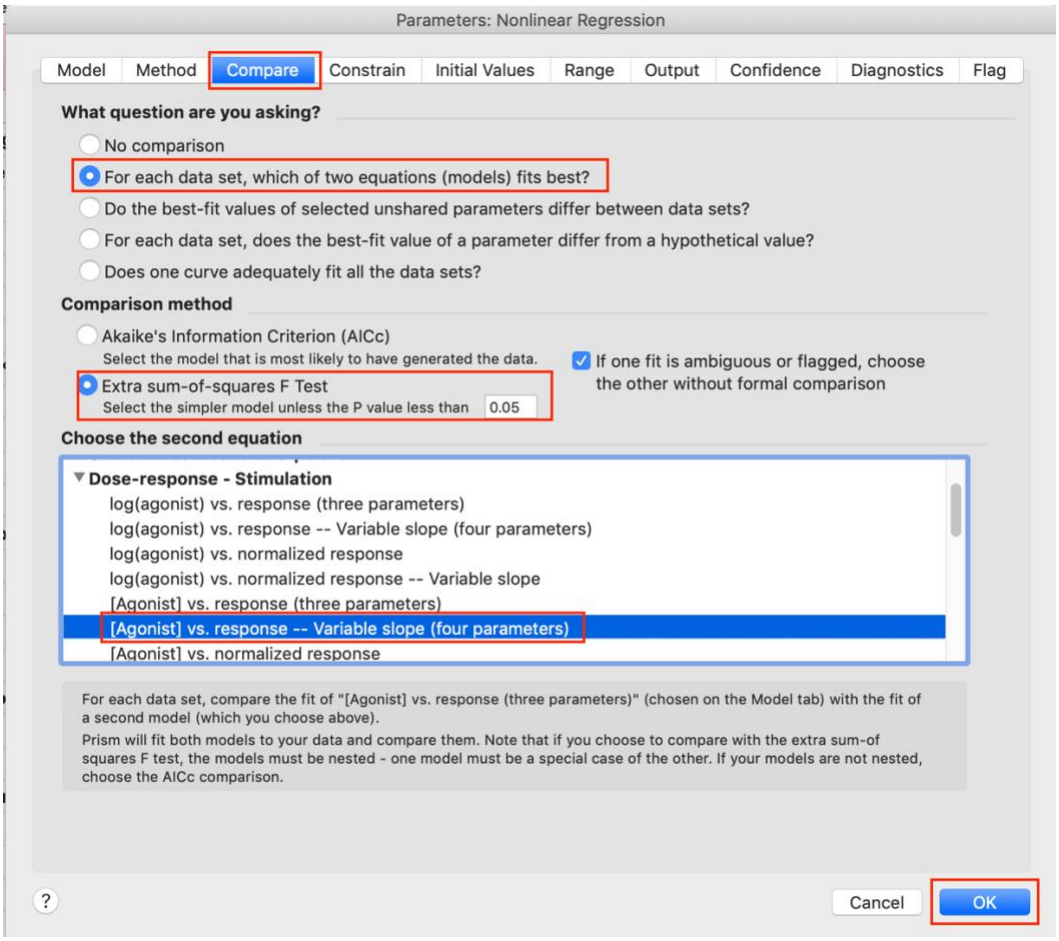
Go to the 3rd tab "**Compare**". Choose "**For each data set, which of two equations (models) fits best?**" as the question we would like to answer; Choose "**Extra sum-of-squares F Test**" OR "**Akaike's information Criterion (AICc)**"as the comparison method; Choose **"[Agonist] vs. response – Variable slop (four parameters)"** as the second equation. **Click "OK".**



Prism offers two approaches to comparing models with different numbers of parameters: Extra sum-of squares F test and Information theory approach Akaike's criterion (AIC):

a) Extra sum-of squares F test

The F test compares the difference in sum-of-squares with the difference you would expect by chance. If the p-value is small, conclude that the simple model is wrong, and accept the more complicated model.

The results of comparing these two models are generated accordingly:

Table of results

Nonlin fit Table of results	A No inhibitor	B Inhibitor
	Y	Y
1 **Comparison of Fits**		
2 Null hypothesis	[Agonist] vs. response (three parameters)	[Agonist] vs. response (three parameters)
3 Alternative hypothesis	[Agonist] vs. response -- Variable slope (four parameters)	[Agonist] vs. response -- Variable slope (four parameters)
4 P value	0.4582	0.5651
5 Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis
6 Preferred model	[Agonist] vs. response (three parameters)	[Agonist] vs. response (three parameters)
7 F (DFn, DFd)	0.5692 (1, 23)	0.3400 (1, 25)

In this case, the p-values are ~0.5 which is larger than the traditional value of 0.05, we cannot reject our null hypothesis that the simpler model is correct.

b) Information theory approach Akaike's criterion (AICc)

This alternative approach is based on information theory, and does not use the traditional "hypothesis testing" statistical paradigm. It determines how well the data supports each model, taking into account both the goodness-of-fit (sum-of-squares) and the number of parameters in the model. The results are expressed as the probability that each model is correct, with the probabilities summing to 100%. If one model is much more likely to be correct than the other (say, 1% vs. 99%), you will want to choose it. If the difference in likelihood is not very big (say, 40% vs. 60%), you will know that either model might be correct, so will want to collect more data.

The AICc results of this model comparison are:

Table of results

Nonlin fit Table of results		A No inhibitor	B Inhibitor
		Y	Y
1	**Comparison of Fits**		
2	Simpler model	[Agonist] vs. response (three parameters)	[Agonist] vs. response (three parameters)
3	Probability it is correct	76.66%	78.16%
4	Alternative model	[Agonist] vs. response -- Variable slope (four parameters)	[Agonist] vs. response -- Variable slope (four parameters)
5	Probability it is correct	23.34%	21.84%
6	Ratio of probabilities	3.285	3.579
7	Preferred model	[Agonist] vs. response (three parameters)	[Agonist] vs. response (three parameters)
8	Difference in AICc	-2.379	-2.550

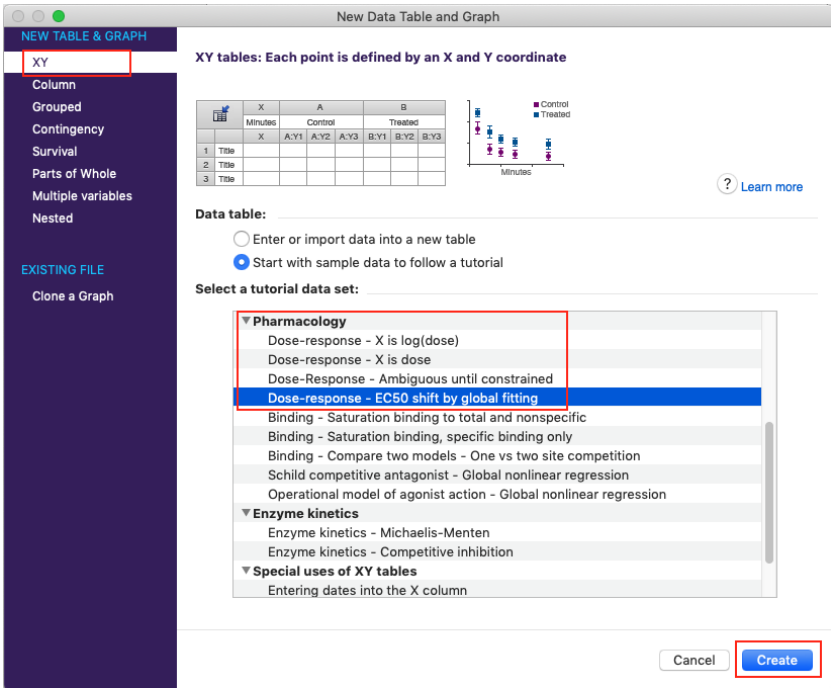
In this case, for the group "No inhibitor", the probability that the model WITHOUT variable slope (76.66%) is correct is higher than the probability that the model WITH variable slope (23.34%) is correct. Therefore, the model WITHOUT variable slope is preferred.

Which approach to choose? F test or AICc?

- If the two models are nested (one model is a simpler case of the other), you may use either the F test or the AIC approach.
- If the models are not nested, then the F test is not valid, so you should choose the information theory approach.

3. Customizing your equation of models

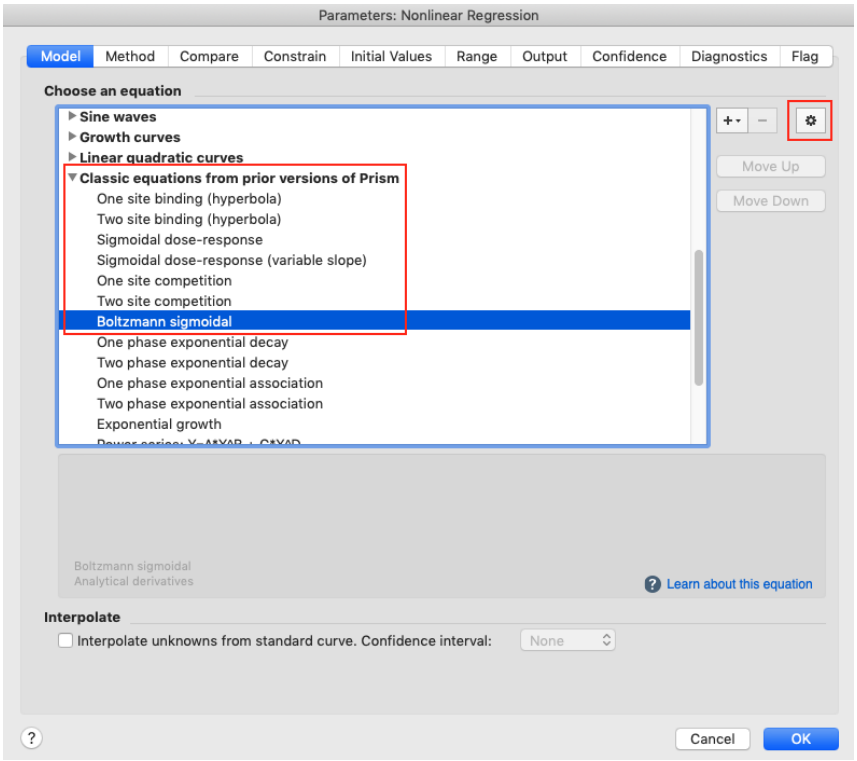
Let's take Boltzmann sigmoidal equation, a classical equation in Prism as an example. Import the **sample data** with XY data table: **Does-response – EC50 shift by global fitting.**



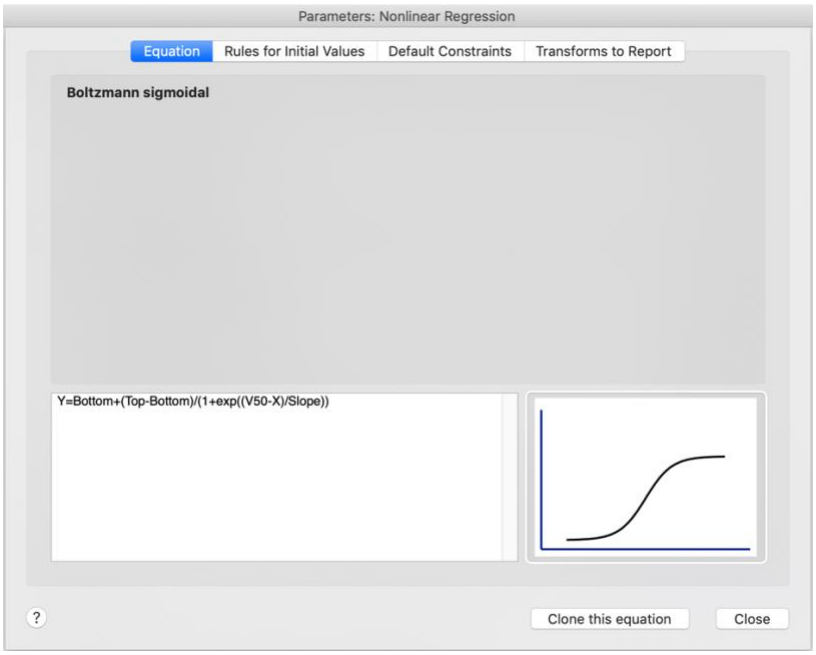
In this case, The X values are the logarithm of the concentration of agonist. The Y values are responses, in duplicate, in two conditions.

		X	Group A		Group B	
		log(agonist)	Control		Treated	
		X	A:Y1	A:Y2	B:Y1	B:Y2
1	Title	-7.5	341	298	295	395
2	Title	-7.0	671	752	616	481
3	Title	-6.5	874	721	362	412
4	Title	-6.0	1000	951	444	700
5	Title	-5.5	1305	1265	882	652
6	Title	-5.0	1254	1351	1354	1089
7	Title	-4.5	1265	1411	1452	1354

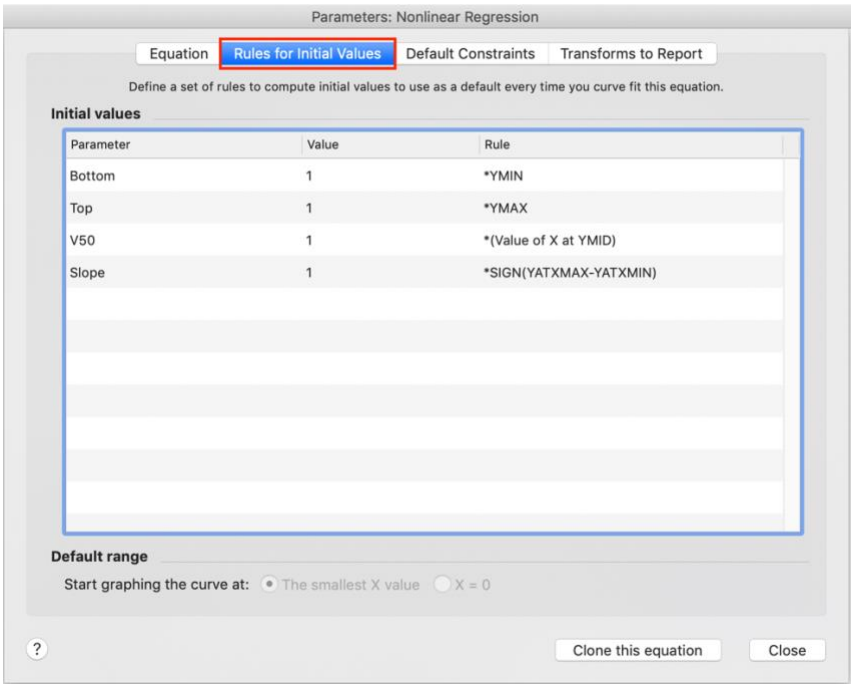
Click "Analyze"; Choose "**Boltzmann sigmoidal**" under the "Classic equations from prior versions of Prism". Then **click the settings button** on the right.



It shows a dialog which presents the Boltzmann sigmoidal equation as well as how this equation looks like in the graph on the right.



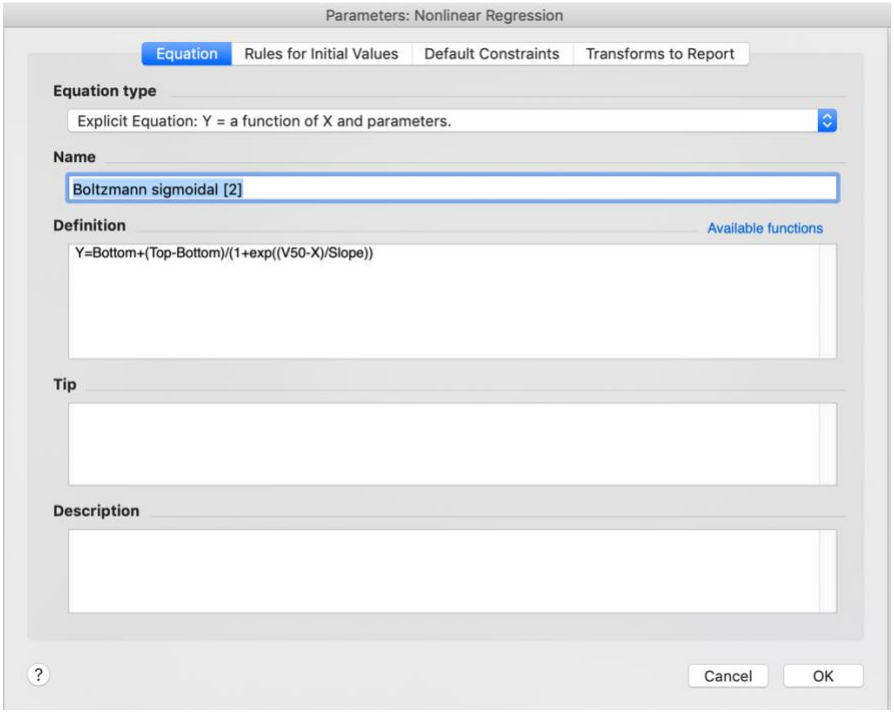
Click the tab "**Rules for Initial Values**" then you could see the initial values defined in the equation.



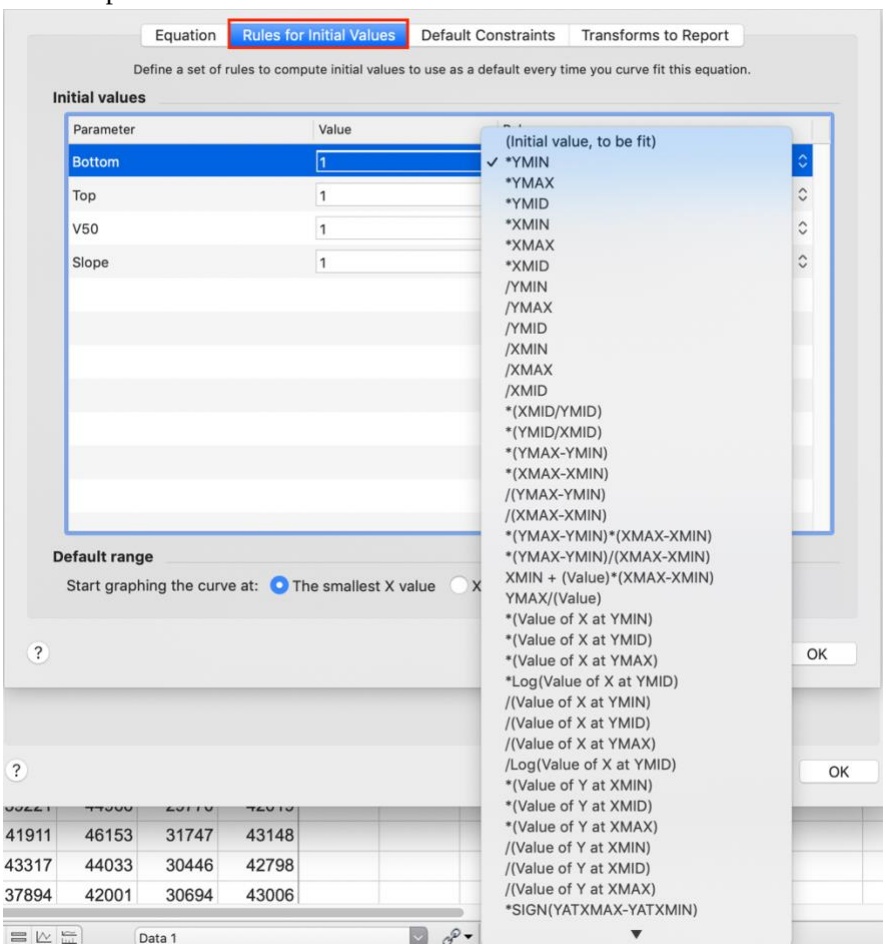
Why should we choose initial values? Nonlinear regression is an iterative procedure. The program must start with estimated initial values for each parameter. It then adjusts these initial values to improve the fit.

In this case, we define the initial Bottom as 1*Ymin, Top as 1*Ymax, V50 as 1*(Value of X at Ymid), and Slope as 1*SIGN(Y at Xmax – Y at Xmin).

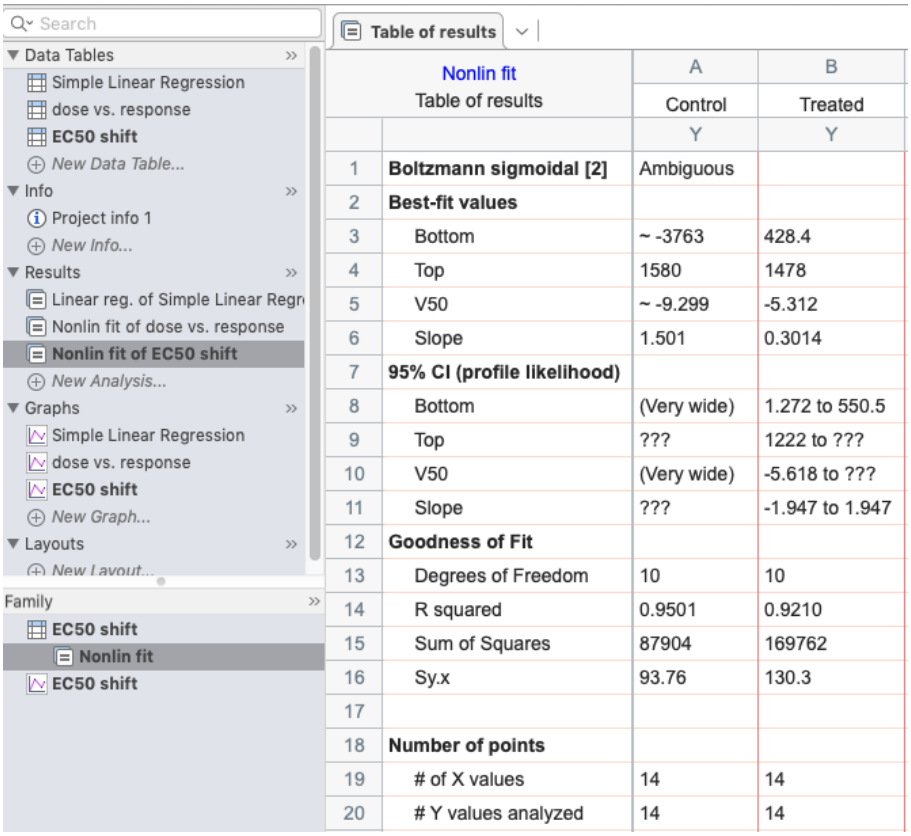
These initial numbers of the equation could be customized by ourselves. Please **click the "Clone the equation"** on the bottom right. Then it pops up a new window for a new customized equation. You could name it and change the formula by your own.



Then **click the tab "Rules for Initial Values"** on the top. You could customize the initial values for each parameter.



Once you **click "OK"**, Prism 8 generates a result table "Nonlin fit of EC50 shift".



Nonlin fit Table of results		A Control Y	B Treated Y
1	**Boltzmann sigmoidal [2]**	Ambiguous	
2	**Best-fit values**		
3	Bottom	~ -3763	428.4
4	Top	1580	1478
5	V50	~ -9.299	-5.312
6	Slope	1.501	0.3014
7	**95% CI (profile likelihood)**		
8	Bottom	(Very wide)	1.272 to 550.5
9	Top	???	1222 to ???
10	V50	(Very wide)	-5.618 to ???
11	Slope	???	-1.947 to 1.947
12	**Goodness of Fit**		
13	Degrees of Freedom	10	10
14	R squared	0.9501	0.9210
15	Sum of Squares	87904	169762
16	Sy.x	93.76	130.3
17			
18	**Number of points**		
19	# of X values	14	14
20	# Y values analyzed	14	14

How to interpret the results?

- **Best-fit values:** the estimate of each parameter. Please note that these estimates are not necessarily equal to those initial values we defined in the equation.
- **95% CI:** Nonlinear regression is an iterative procedure. The program must start with estimated initial values for each parameter. It then adjusts these initial values to improve the fit.
- **Goodness of Fit:**
    - R squared: quantifies goodness of fit. Higher values indicate that the model fits the data better.
    - Sum of Squares: This is useful if you want to compare Prism with another program, or compare two fits manually. Otherwise, the value is not very helpful.

Conclusions

1. BCBB keeps updating this file for more examples and explanations. Please feel free to watch/star our Github repository BCBB Prism Lab: Curve Fitting to be notified for update.
2. If you have any further question, please let me know qinlu.wang@nih.gov.

Reference:

Prism 8 Curve Fitting guide