Pharmechanics

GraphPad

Kinase inhibitor residence time curve fitting

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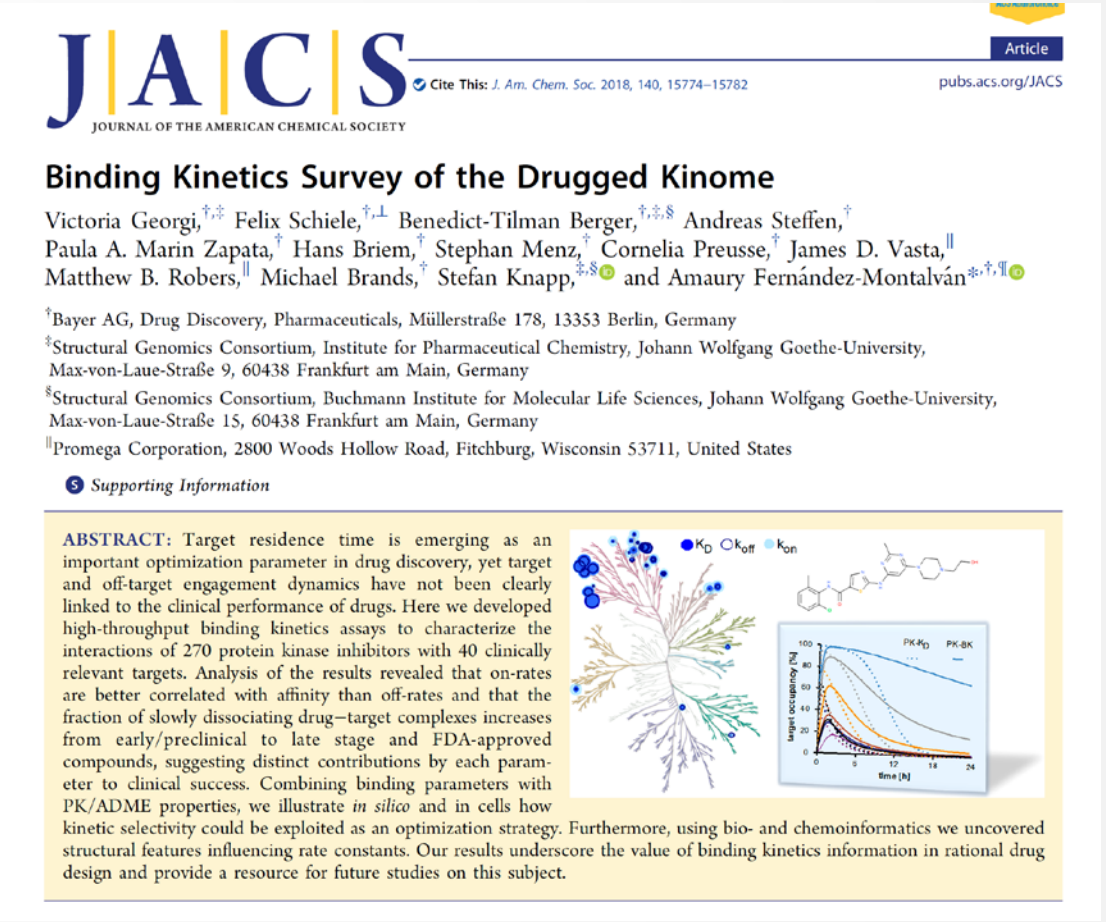
Background

- Binding kinetics of kinase inhibitors is of great interest in drug discovery.
- The inhibitor residence time is frequently measured using a washout protocol:

  Methods Mol Biol 2019, 1888: 45-71 (Figs 8 & 13)
  Promega webinar (slides 20 & 21)
  Aurelia Bioscience examples



JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Article
Cite This: J. Am. Chem. Soc. 2018, 140, 15774−15782
pubs.acs.org/JACS

Binding Kinetics Survey of the Drugged Kinome

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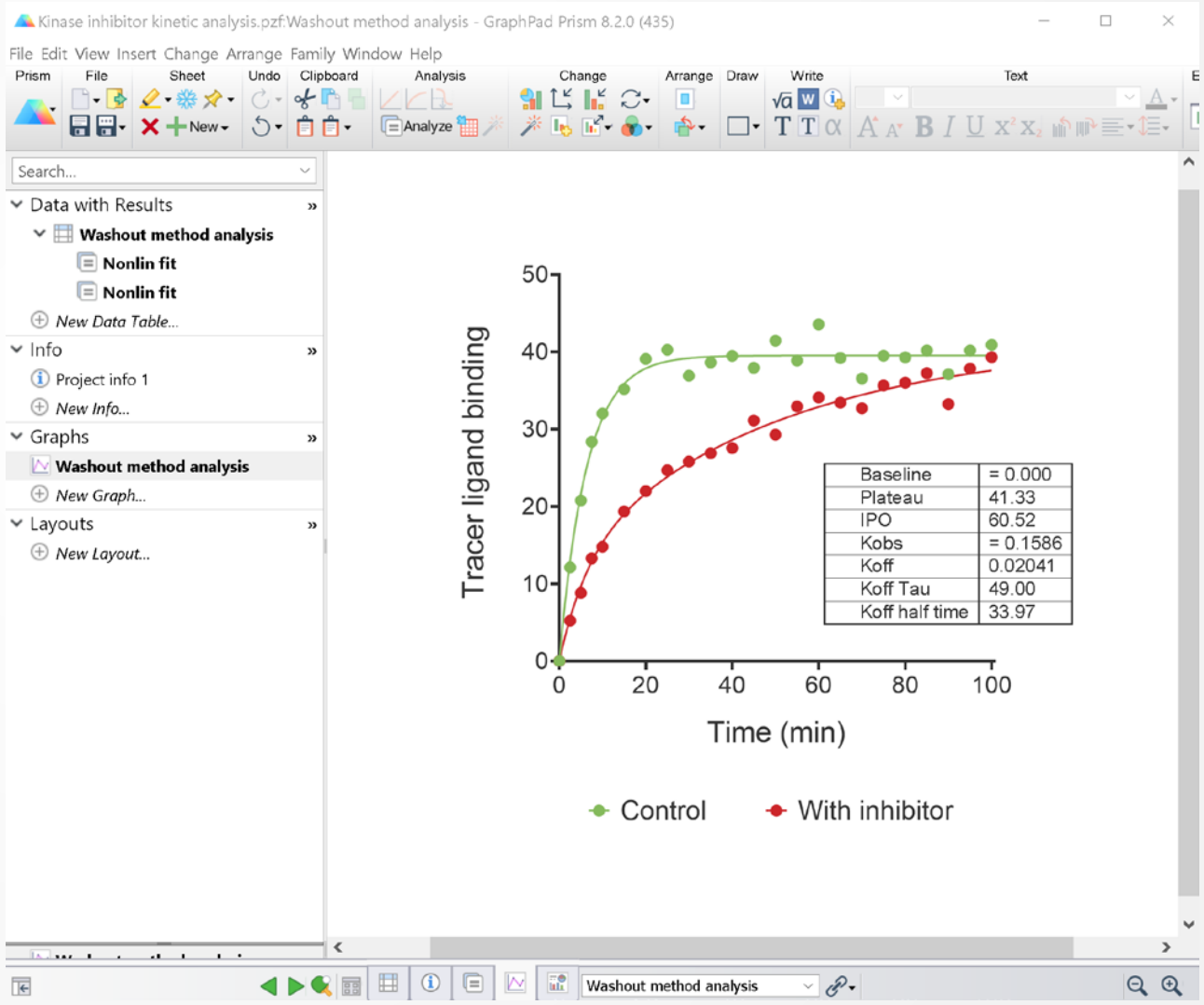
Supporting Information

ABSTRACT: Target residence time is emerging as an important optimization parameter in drug discovery, yet target and off-target engagement dynamics have not been clearly linked to the clinical performance of drugs. Here we developed high-throughput binding kinetics assays to characterize the interactions of 270 protein kinase inhibitors with 40 clinically relevant targets. Analysis of the results revealed that on-rates are better correlated with affinity than off-rates and that the fraction of slowly dissociating drug−target complexes increases from early/preclinical to late stage and FDA-approved compounds, suggesting distinct contributions by each parameter to clinical success. Combining binding parameters with PK/ADME properties, we illustrate *in silico* and in cells how kinetic selectivity could be exploited as an optimization strategy. Furthermore, using bio- and chemoinformatics we uncovered structural features influencing rate constants. Our results underscore the value of binding kinetics information in rational drug design and provide a resource for future studies on this subject.

New regression model

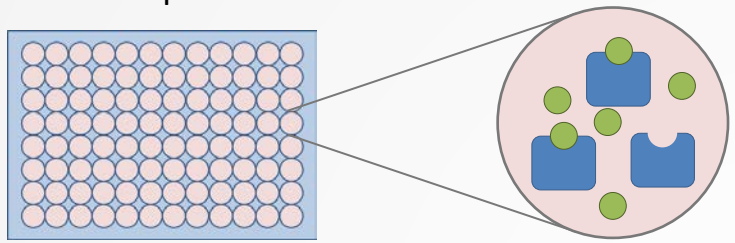
- A new equation for fitting the washout method data, that estimates the residence time, has been loaded into GraphPad Prism in a custom template designed by Pharmechanics.

- The template can be obtained from here.



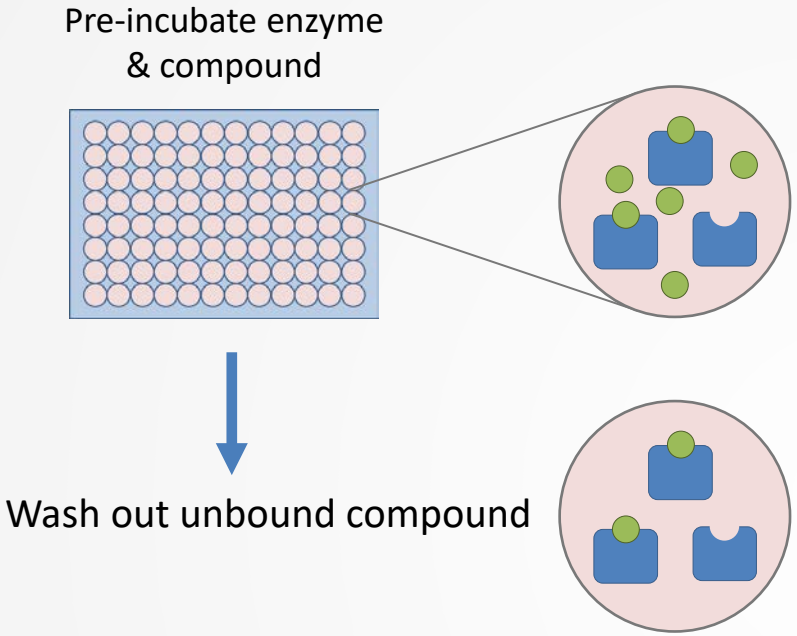
Washout experiment protocol

Pre-incubate enzyme
& compound



See J Recept Signal Transduct Res 2009, 29: 84-93

Washout experiment protocol



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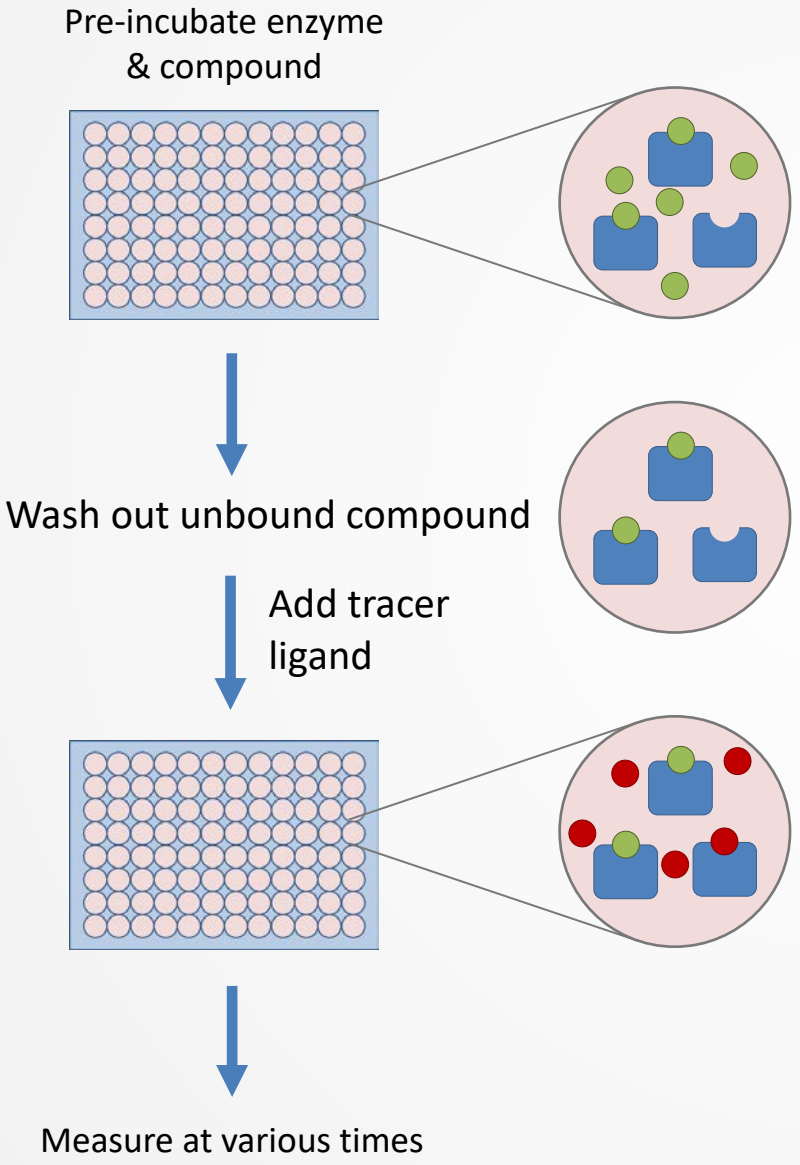
Washout experiment protocol



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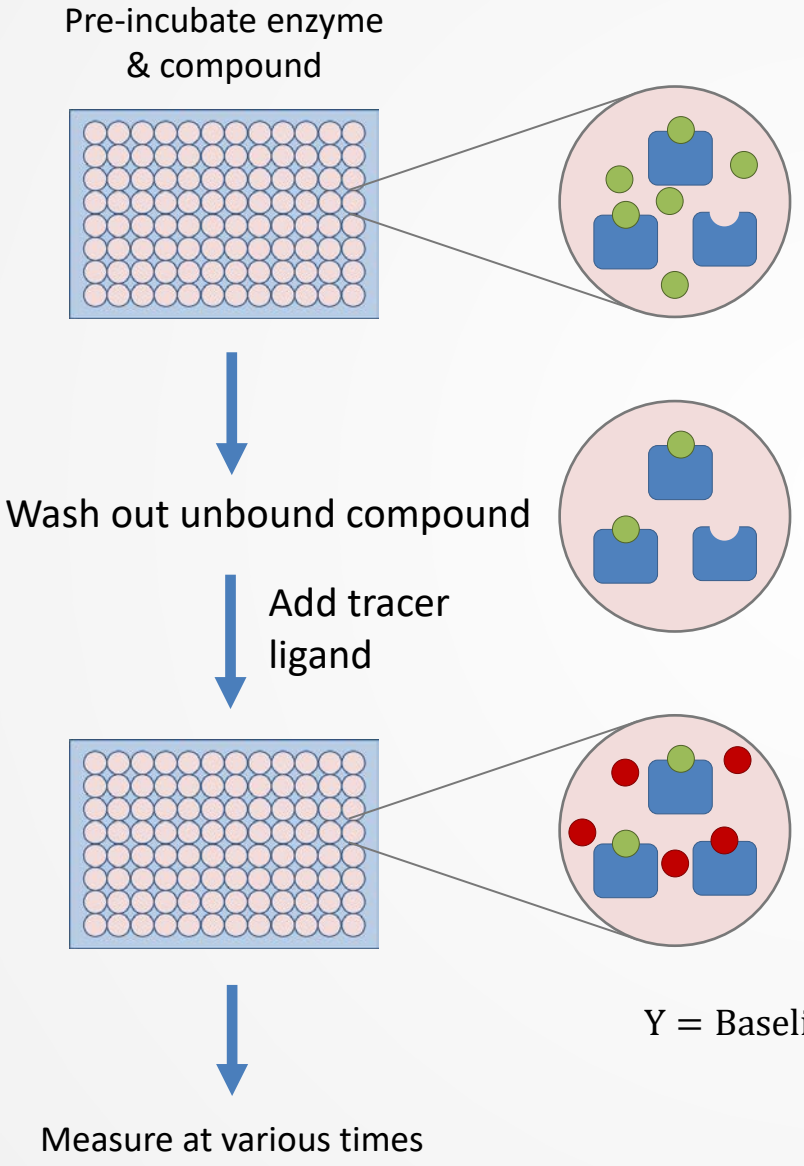
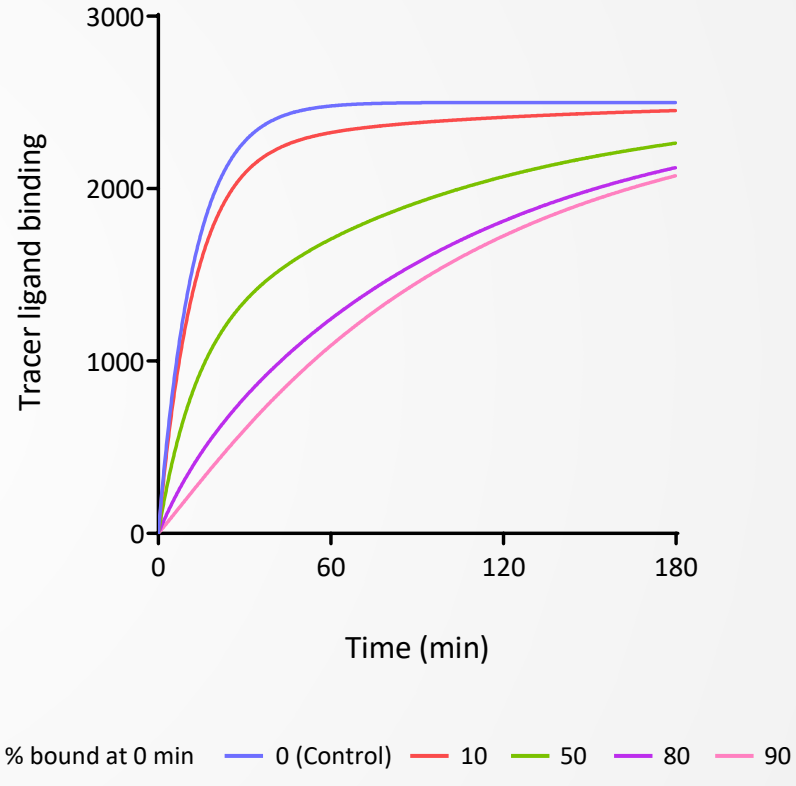
Washout experiment protocol





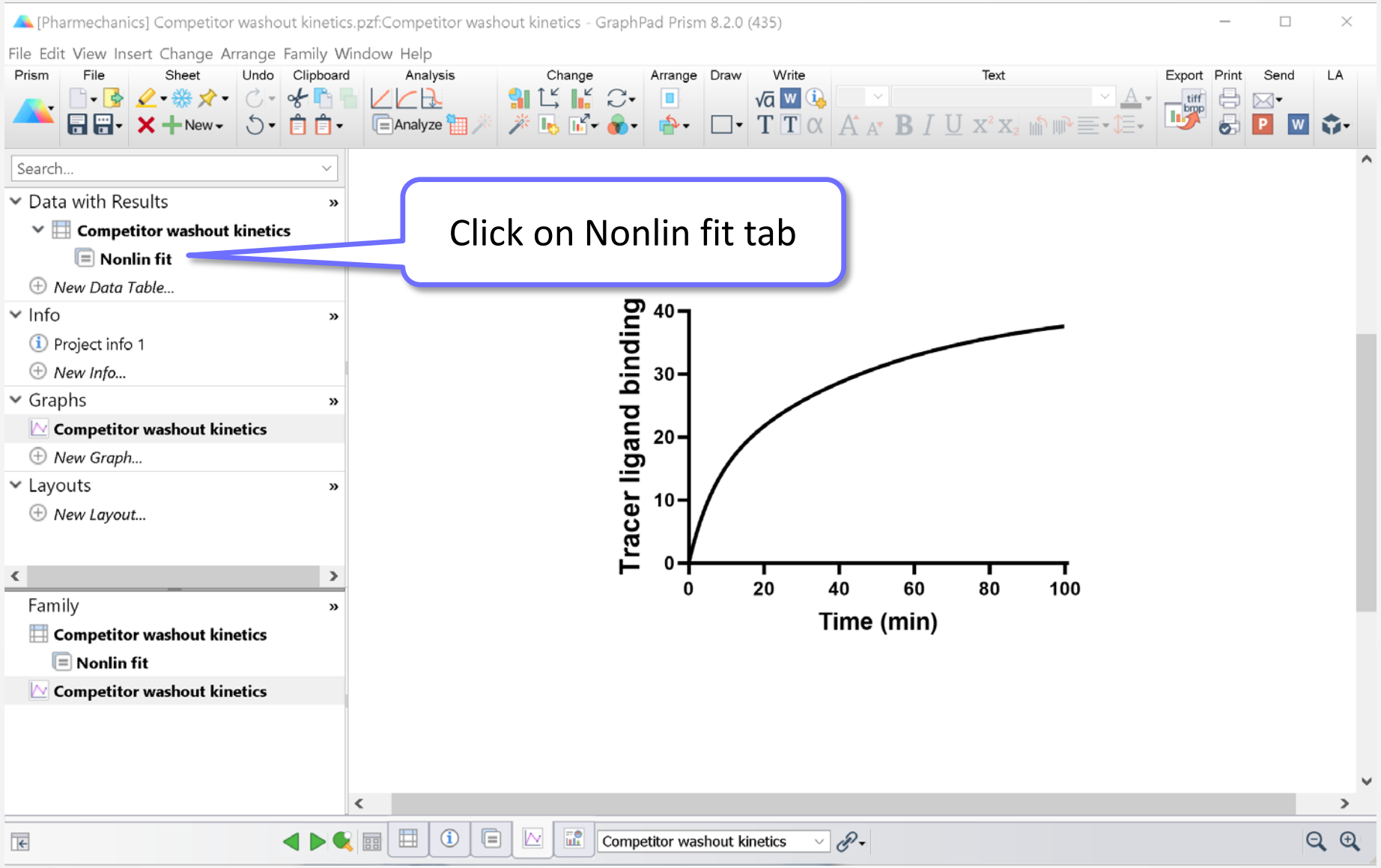
$$Y = \text{Baseline} + (\text{Plateau} - \text{baseline}) \times \left(1 - \left(1 - \frac{\text{IPO}.k_{obs}}{k_{obs} - k_{off}}\right) e^{-k_{obs}.t} - \frac{\text{IPO}.k_{obs}}{k_{obs} - k_{off}} e^{-k_4.t}\right)$$

See J Recept Signal Transduct Res 2009, 29: 84-93

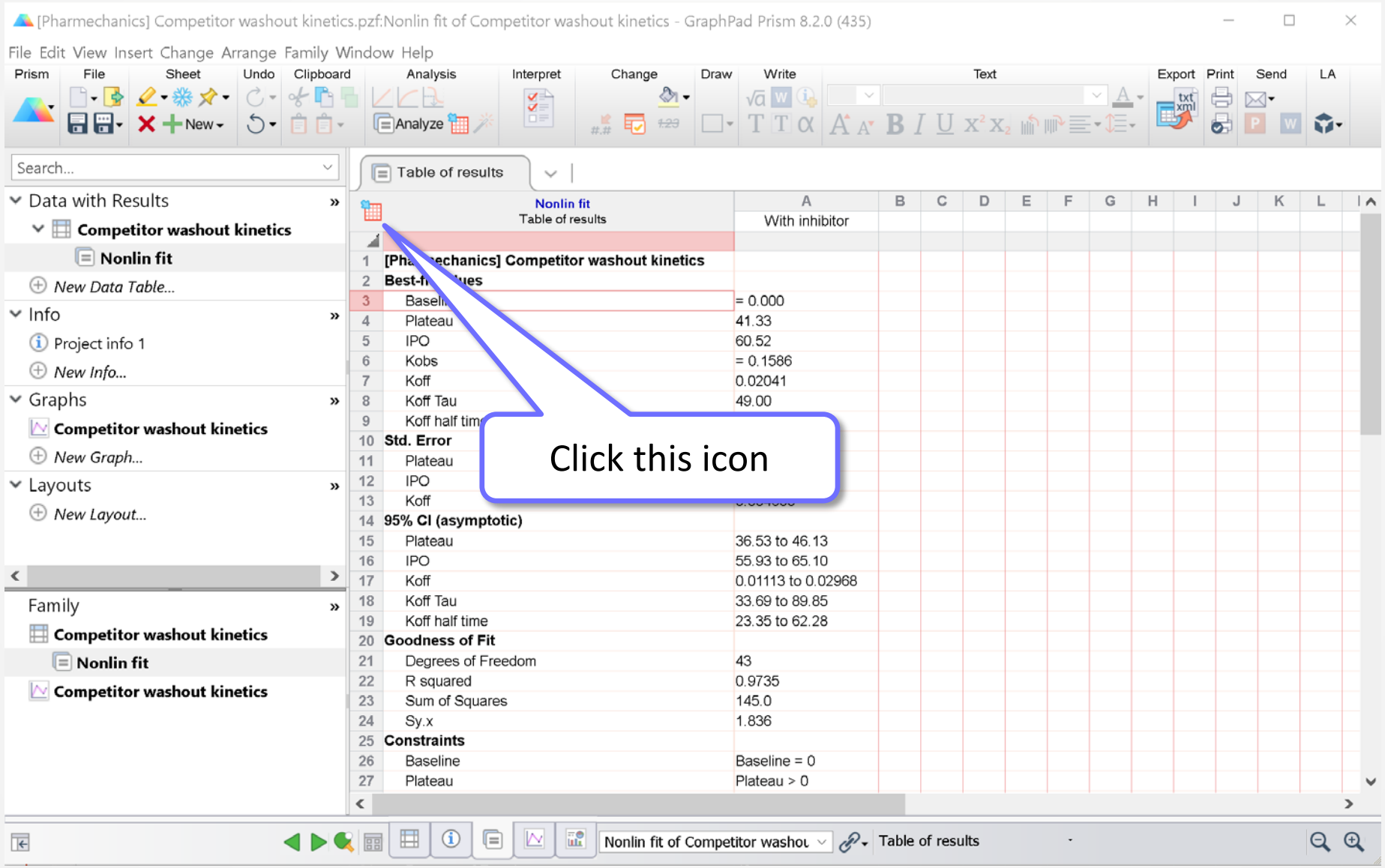
Loading the new equation into Prism

- Download “[Pharmechanics] Competitor washout kinetics” from here.
- Open file and follow instructions on the following slides.

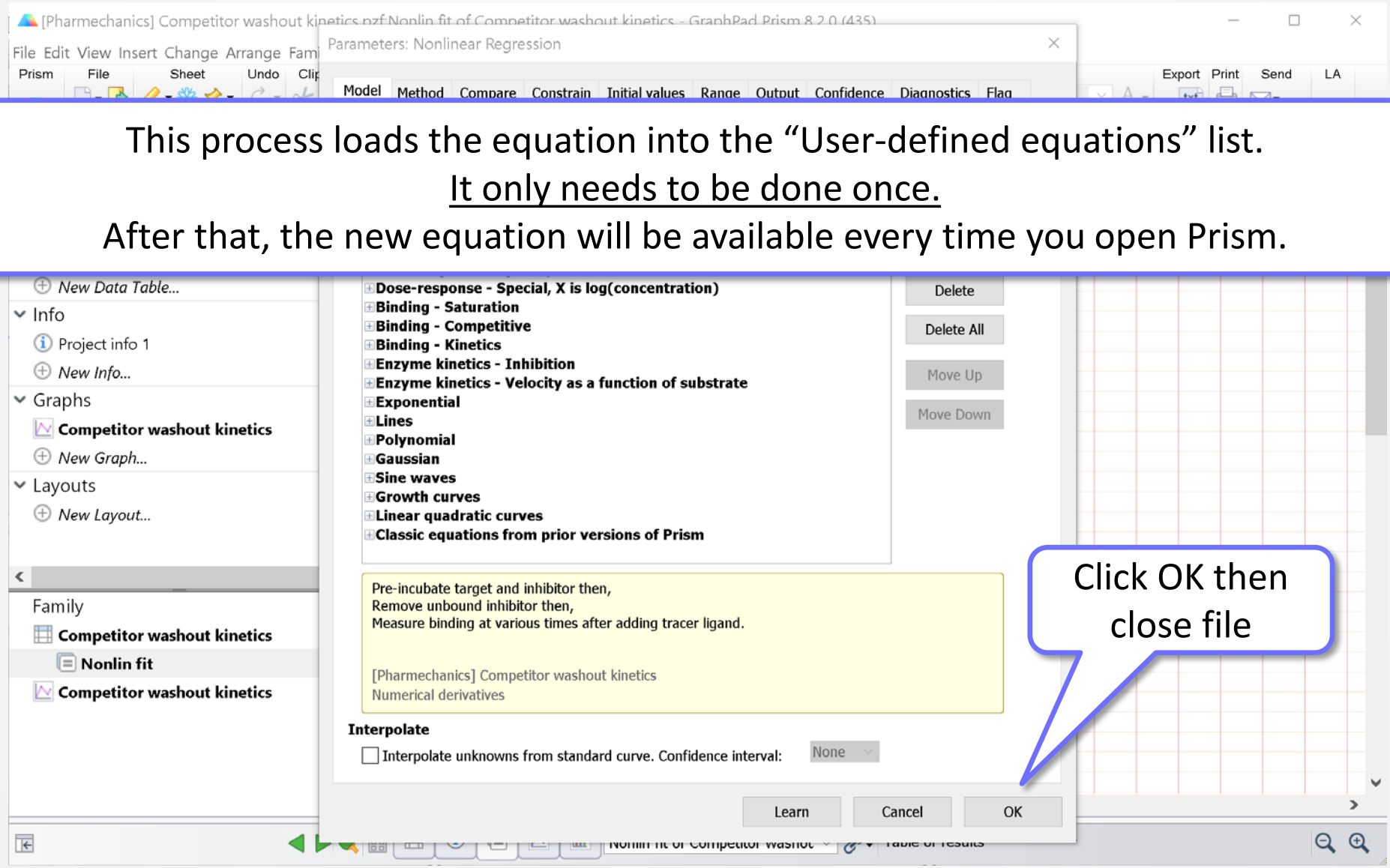
Loading the new equation into Prism



Loading the new equation into Prism



Loading the new equation into Prism



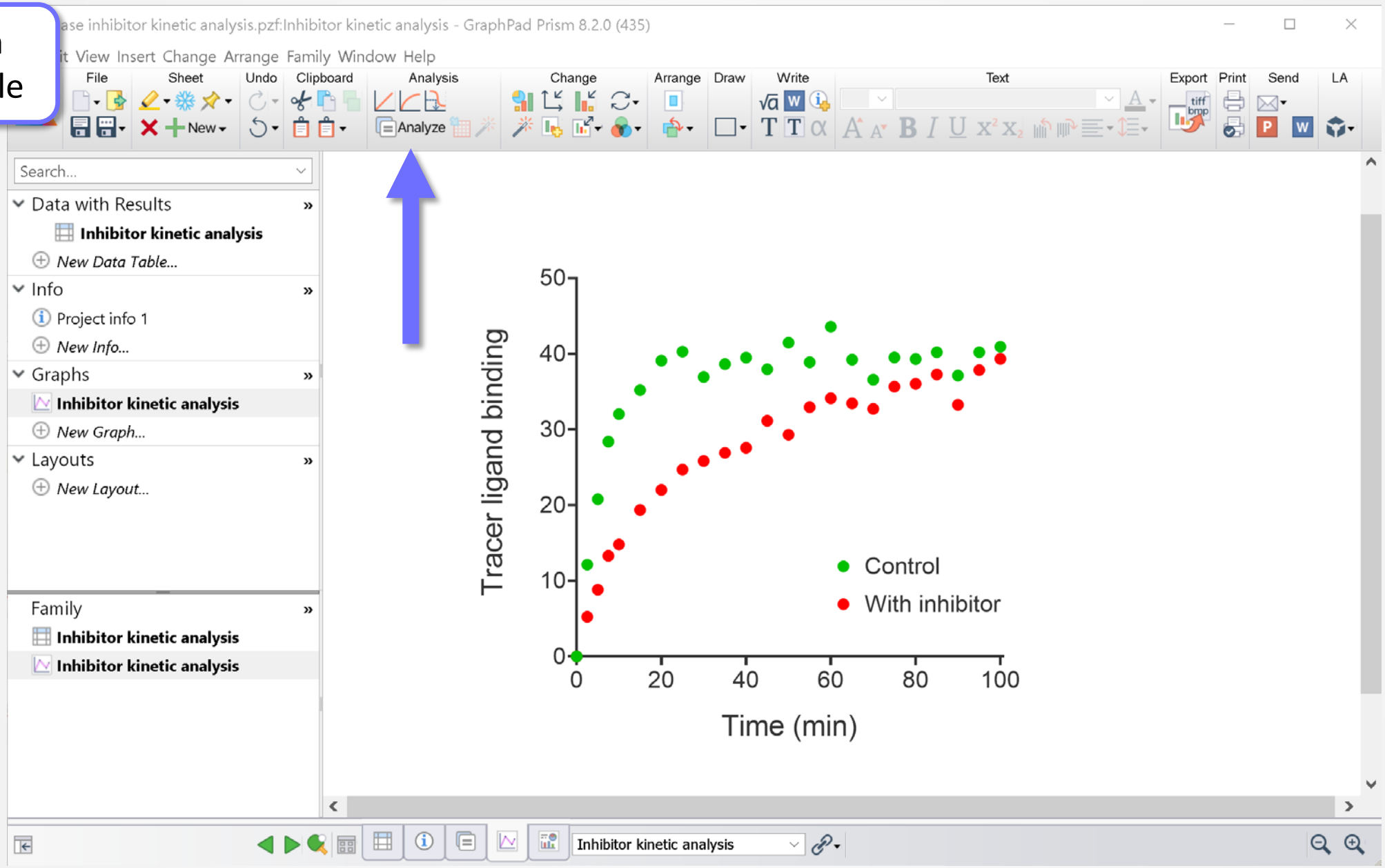
Data analysis Step 1: Control

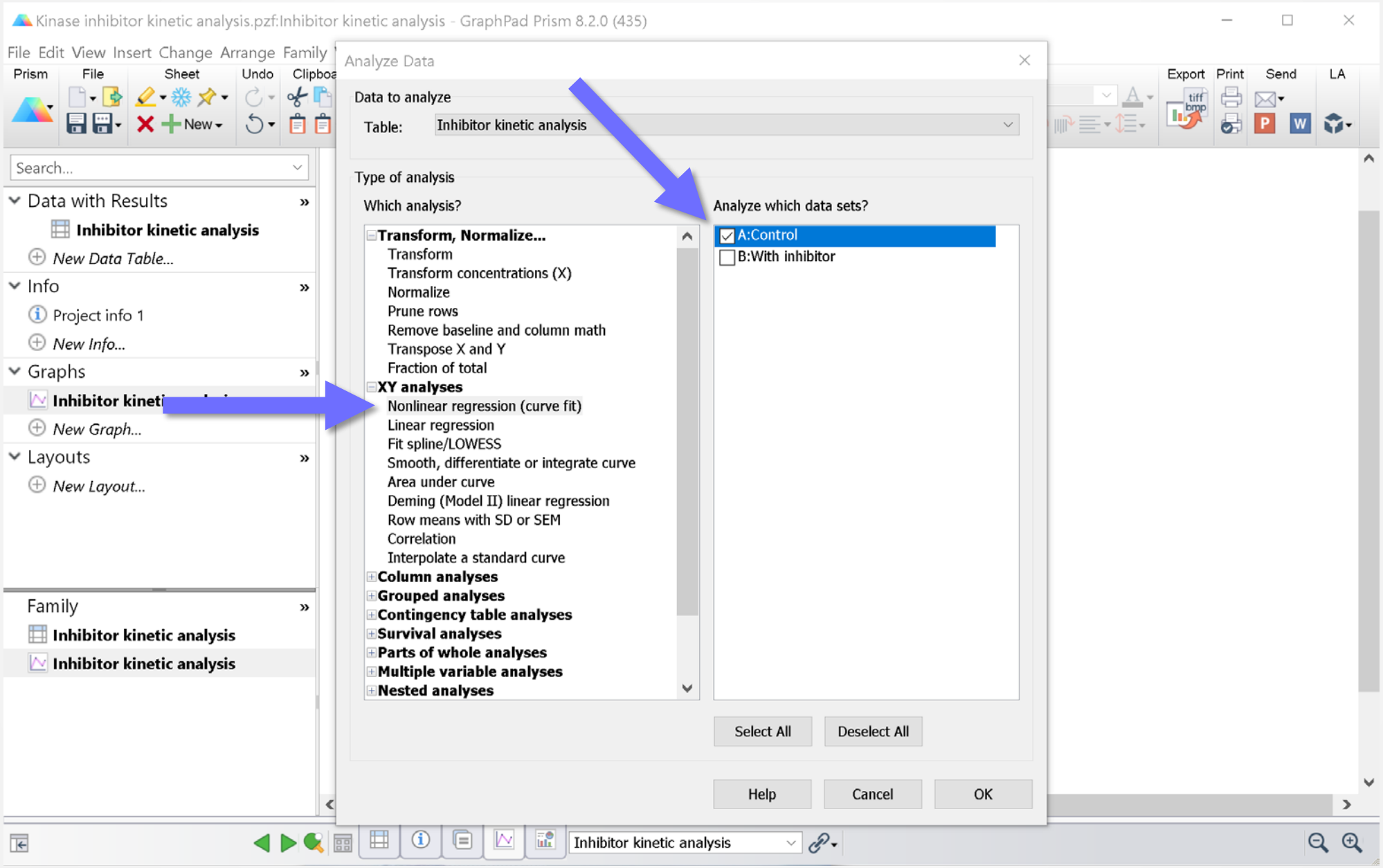
Fit the control data (no inhibitor) to determine the observed association rate constant of the tracer.

Use built-in “One phase association” equation.

See screen shots on next slides.

Open your file

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Inhibitor kinetic analysis
Analyze Data
Data to analyze
Table:
Inhibitor kinetic analysis
Type of analysis
Which analysis?
Analyze which data sets?
Transform, Normalize...
Transform
Transform concentrations (X)
Normalize
Prune rows
Remove baseline and column math
Transpose X and Y
Fraction of total
XY analyses
Nonlinear regression (curve fit)
Linear regression
Fit spline/LOWESS
Smooth, differentiate or integrate curve
Area under curve
Deming (Model II) linear regression
Row means with SD or SEM
Correlation
Interpolate a standard curve
Column analyses
Grouped analyses
Contingency table analyses
Survival analyses
Parts of whole analyses
Multiple variable analyses
Nested analyses
A:Control
B:With inhibitor
Select All
Deselect All
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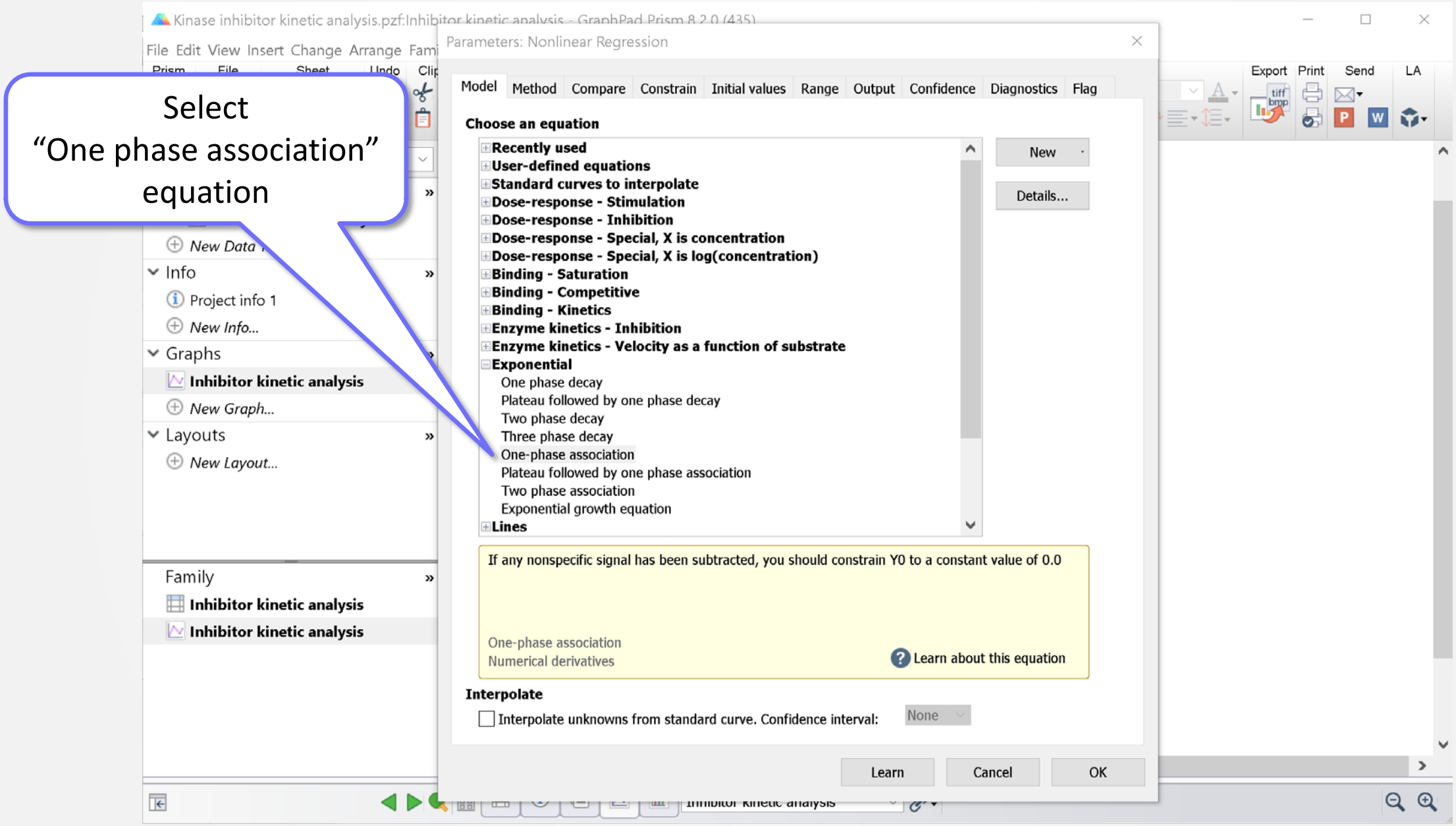
OK
Inhibitor kinetic analysis


Select
“One phase association”
equation
Parameters: Nonlinear Regression
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Choose an equation
Recently used
User-defined equations
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
One phase decay
Plateau followed by one phase decay
Two phase decay
Three phase decay
One-phase association
Plateau followed by one phase association
Two phase association
Exponential growth equation
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Details...
If any nonspecific signal has been subtracted, you should constrain Y0 to a constant value of 0.0
One-phase association
Numerical derivatives
Learn about this equation
Interpolate
Interpolate unknowns from standard curve. Confidence interval:
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Learn
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OK
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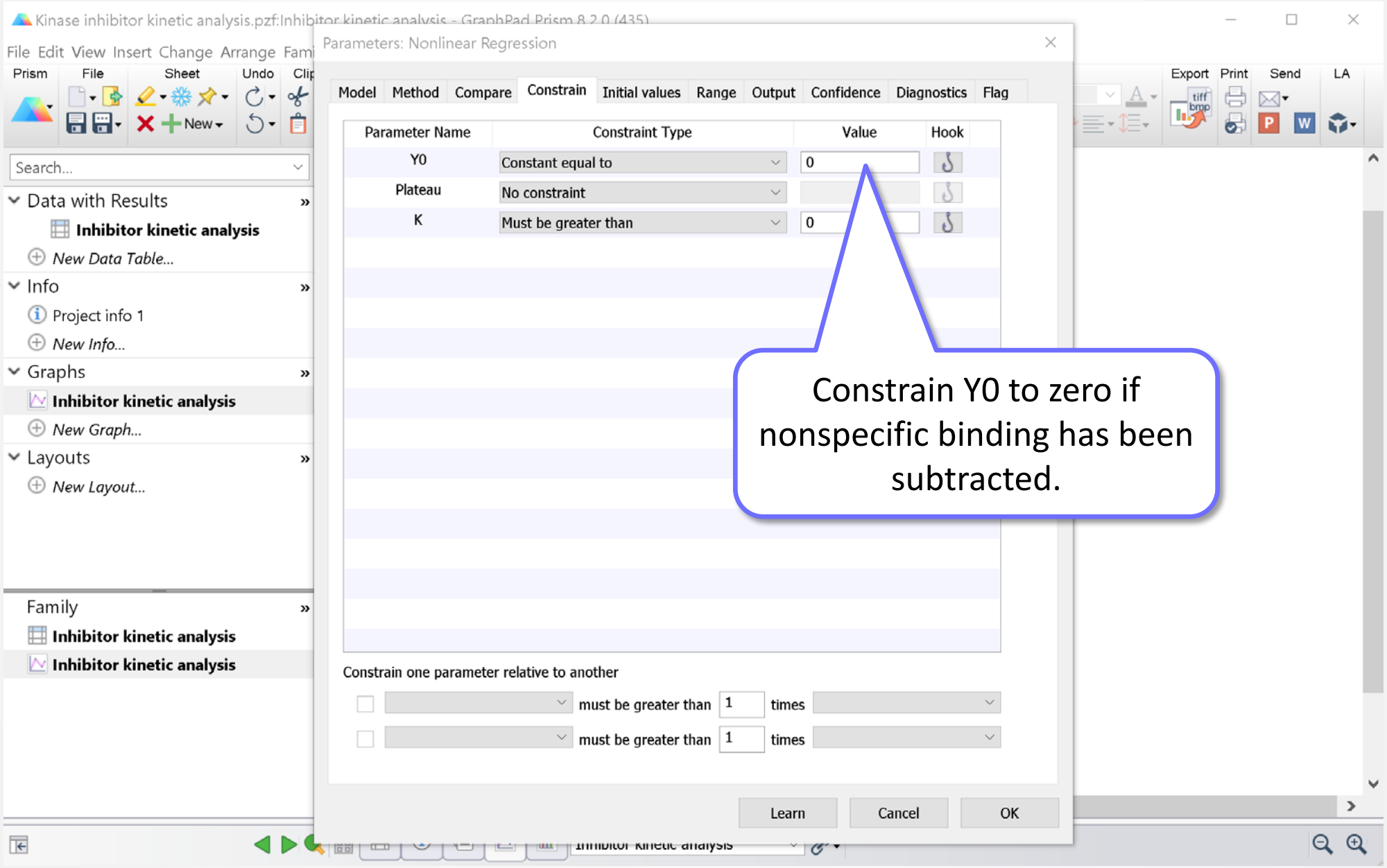
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Constraint Type
Value
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Y0
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Plateau
No constraint
K
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Constrain one parameter relative to another
must be greater than
1
times
must be greater than
1
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OK
Constrain Y0 to zero if nonspecific binding has been subtracted.
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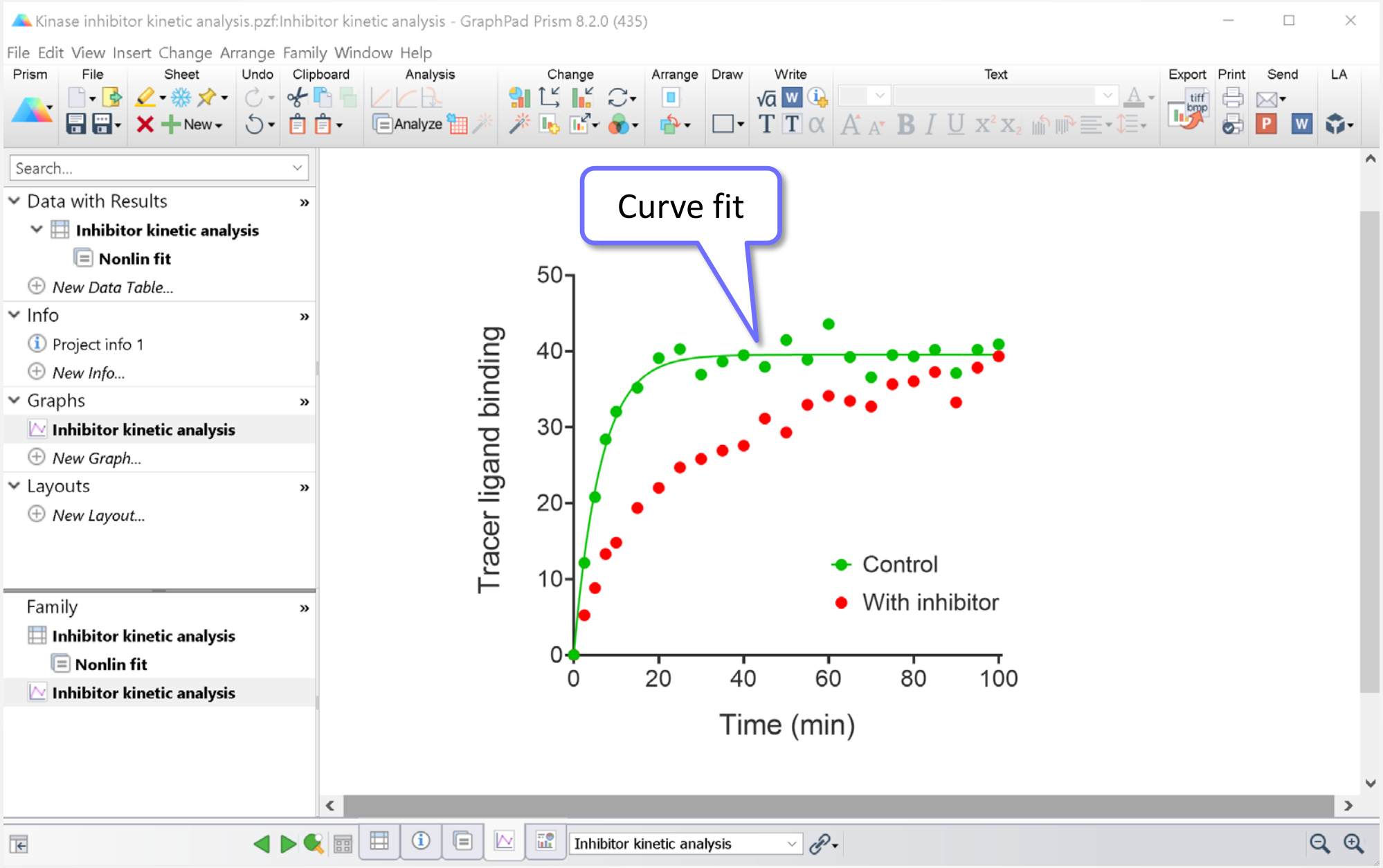
Inhibitor kinetic analysis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Tracer ligand binding
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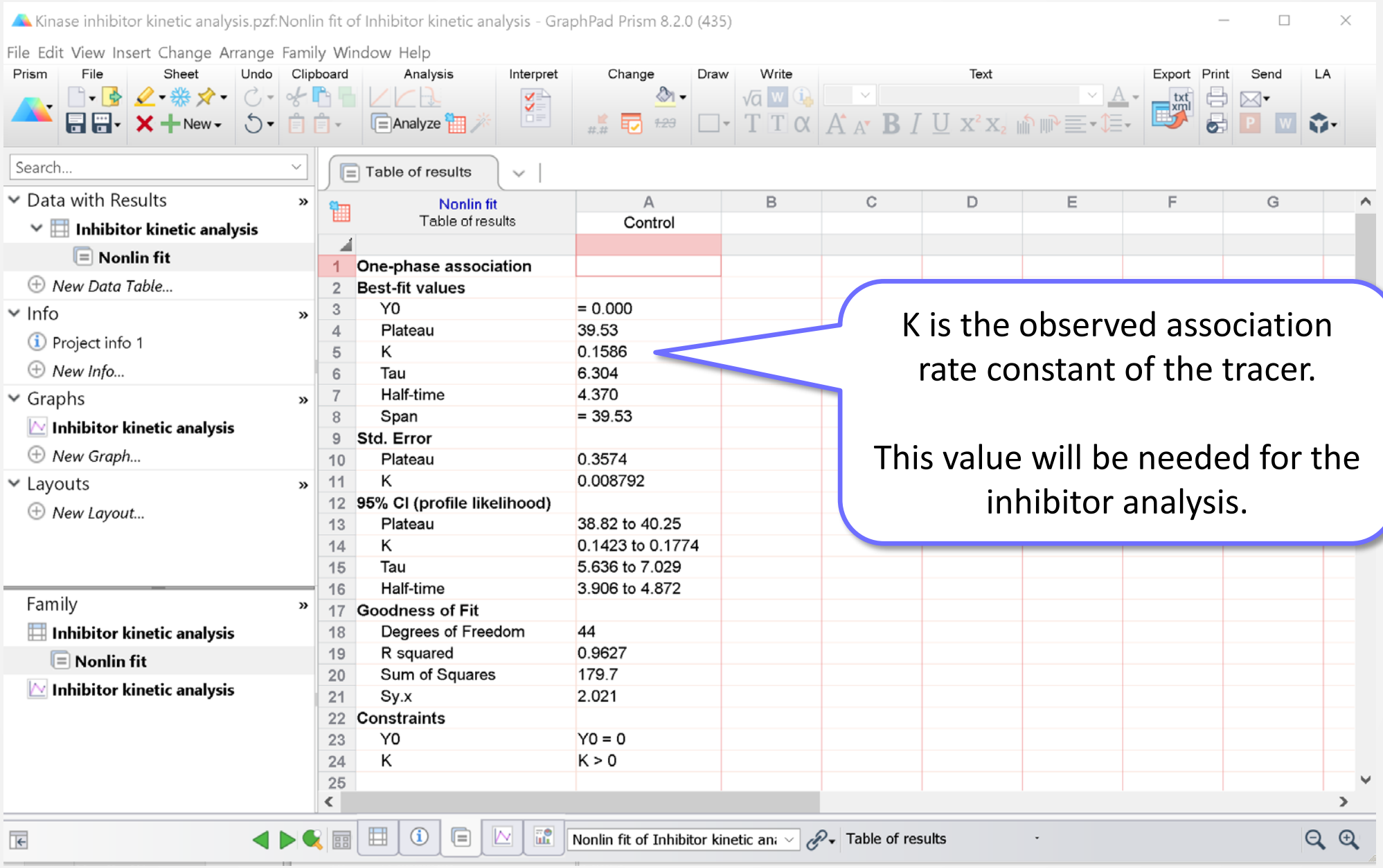
Inhibitor kinetic analysis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Nonlin fit
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Table of results
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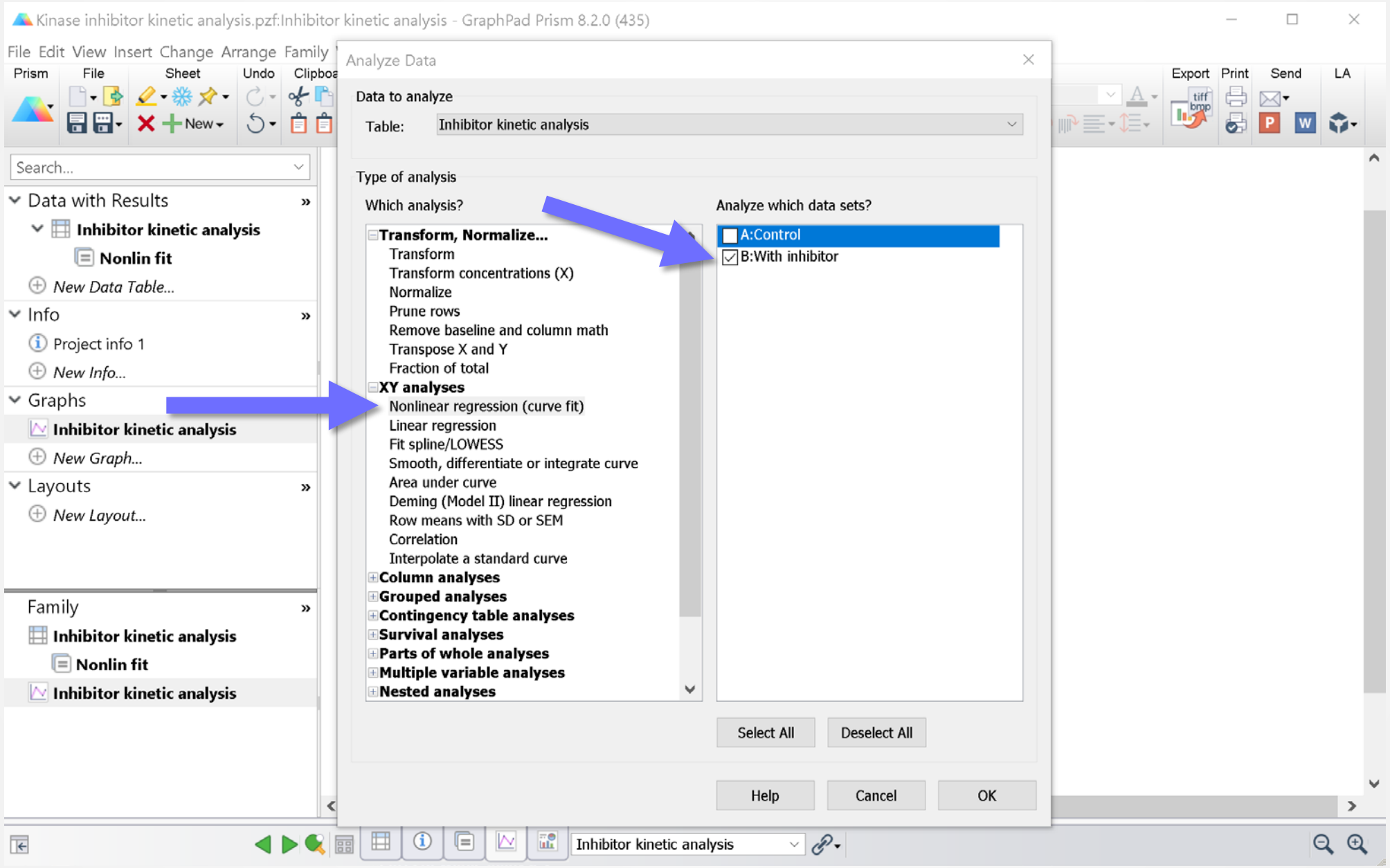
Table of results
A
Control
B C D E F G
1 One-phase association
2 Best-fit values
3 Y0 = 0.000
4 Plateau 39.53
5 K 0.1586
6 Tau 6.304
7 Half-time 4.370
8 Span = 39.53
9 Std. Error
10 Plateau 0.3574
11 K 0.008792
12 95% CI (profile likelihood)
13 Plateau 38.82 to 40.25
14 K 0.1423 to 0.1774
15 Tau 5.636 to 7.029
16 Half-time 3.906 to 4.872
17 Goodness of Fit
18 Degrees of Freedom 44
19 R squared 0.9627
20 Sum of Squares 179.7
21 Sy.x 2.021
22 Constraints
23 Y0 Y0 = 0
24 K K > 0
25
Nonlin fit of Inhibitor kinetic ana
Table of results
K is the observed association rate constant of the tracer.
This value will be needed for the inhibitor analysis.

Data analysis Step 2: Inhibitor

Fit the inhibitor data to determine the dissociation rate constant of the inhibitor.

Use User-defined equation
“[Pharmechanics] Competitor washout kinetics”

See screen shots on next slides.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Inhibitor kinetic analysis
Nonlin fit
Inhibitor kinetic analysis
Analyze Data
Data to analyze
Table:
Inhibitor kinetic analysis
Type of analysis
Which analysis?
Analyze which data sets?
Transform, Normalize...
Transform
Transform concentrations (X)
Normalize
Prune rows
Remove baseline and column math
Transpose X and Y
Fraction of total
XY analyses
Nonlinear regression (curve fit)
Linear regression
Fit spline/LOWESS
Smooth, differentiate or integrate curve
Area under curve
Deming (Model II) linear regression
Row means with SD or SEM
Correlation
Interpolate a standard curve
Column analyses
Grouped analyses
Contingency table analyses
Survival analyses
Parts of whole analyses
Multiple variable analyses
Nested analyses
A:Control
B:With inhibitor
Select All
Deselect All
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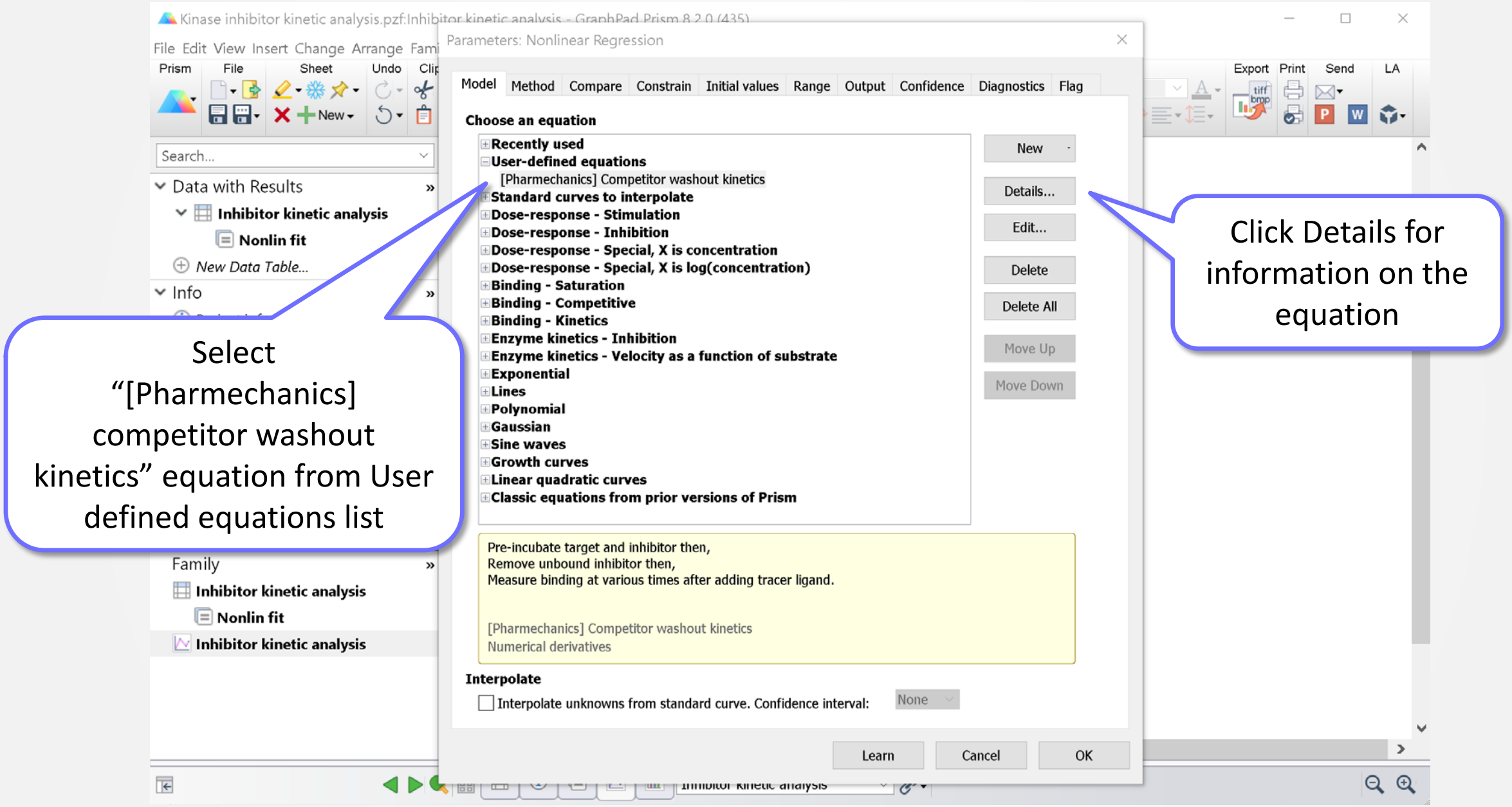
OK
Inhibitor kinetic analysis


Parameters: Nonlinear Regression
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[Pharmechanics] Competitor washout kinetics
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
Linear quadratic curves
Classic equations from prior versions of Prism
New
Details...
Edit...
Delete
Delete All
Move Up
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Pre-incubate target and inhibitor then,
Remove unbound inhibitor then,
Measure binding at various times after adding tracer ligand.
[Pharmechanics] Competitor washout kinetics
Numerical derivatives
Interpolate
Interpolate unknowns from standard curve. Confidence interval:
None
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Select "[Pharmechanics] competitor washout kinetics" equation from User defined equations list
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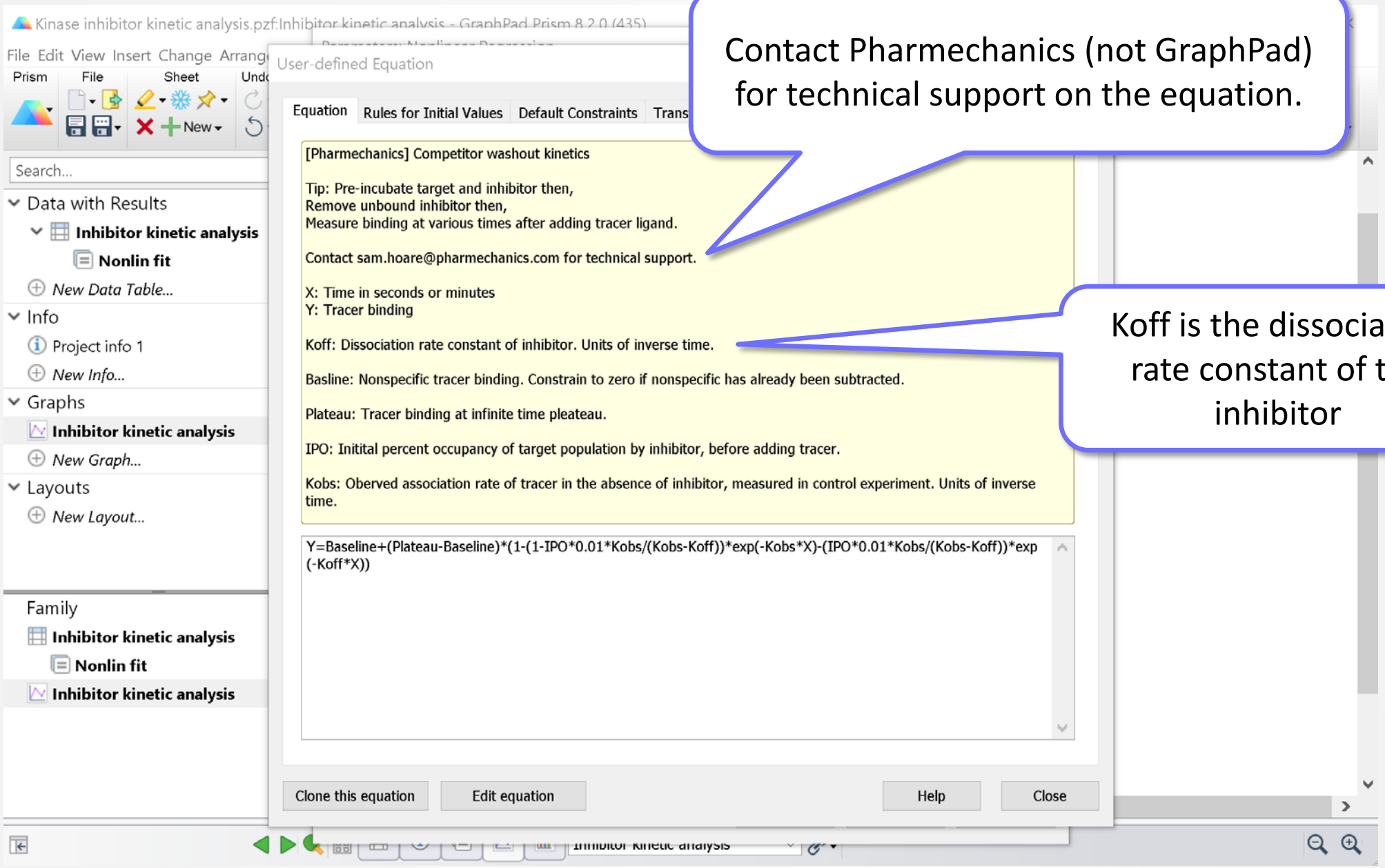
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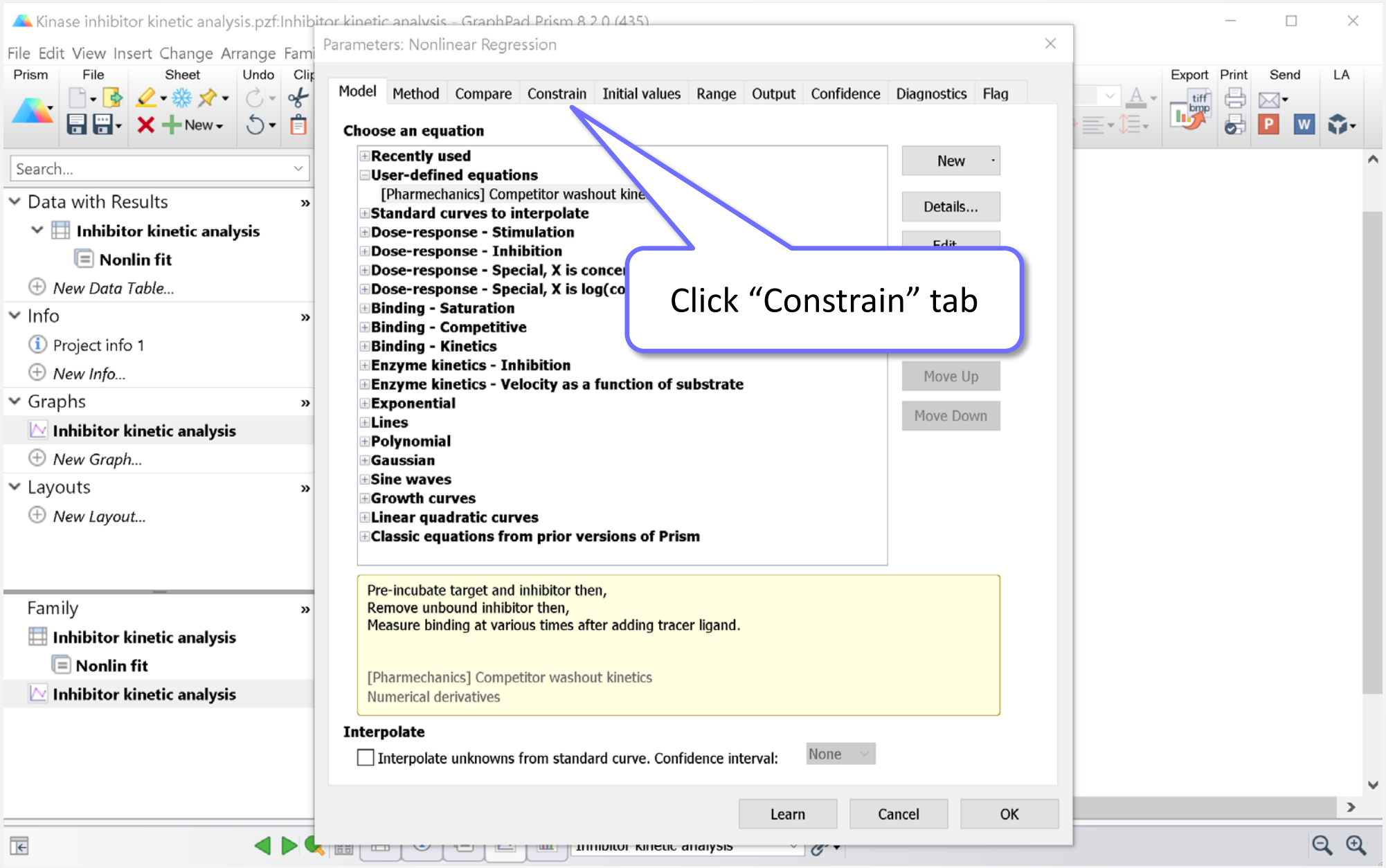
Nonlin fit
Inhibitor kinetic analysis
User-defined Equation
Equation
Rules for Initial Values
Default Constraints
Trans
[Pharmechanics] Competitor washout kinetics
Tip: Pre-incubate target and inhibitor then,
Remove unbound inhibitor then,
Measure binding at various times after adding tracer ligand.
Contact sam.hoare@pharmechanics.com for technical support.
X: Time in seconds or minutes
Y: Tracer binding
Koff: Dissociation rate constant of inhibitor. Units of inverse time.
Basline: Nonspecific tracer binding. Constrain to zero if nonspecific has already been subtracted.
Plateau: Tracer binding at infinite time pleateau.
IPO: Initital percent occupancy of target population by inhibitor, before adding tracer.
Kobs: Oberved association rate of tracer in the absence of inhibitor, measured in control experiment. Units of inverse time.
Y=Baseline+(Plateau-Baseline)*(1-(1-IPO*0.01*Kobs/(Kobs-Koff))*exp(-Kobs*X)-(IPO*0.01*Kobs/(Kobs-Koff))*exp(-Koff*X))
Clone this equation
Edit equation
Help
Close
Contact Pharmechanics (not GraphPad) for technical support on the equation.
Koff is the dissociation rate constant of the inhibitor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Enzyme kinetics - Inhibition
Enzyme kinetics - Velocity as a function of substrate
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Growth curves
Linear quadratic curves
Classic equations from prior versions of Prism
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Details...
Edit...
Move Up
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Pre-incubate target and inhibitor then,
Remove unbound inhibitor then,
Measure binding at various times after adding tracer ligand.
[Pharmechanics] Competitor washout kinetics
Numerical derivatives
Interpolate
Interpolate unknowns from standard curve. Confidence interval:
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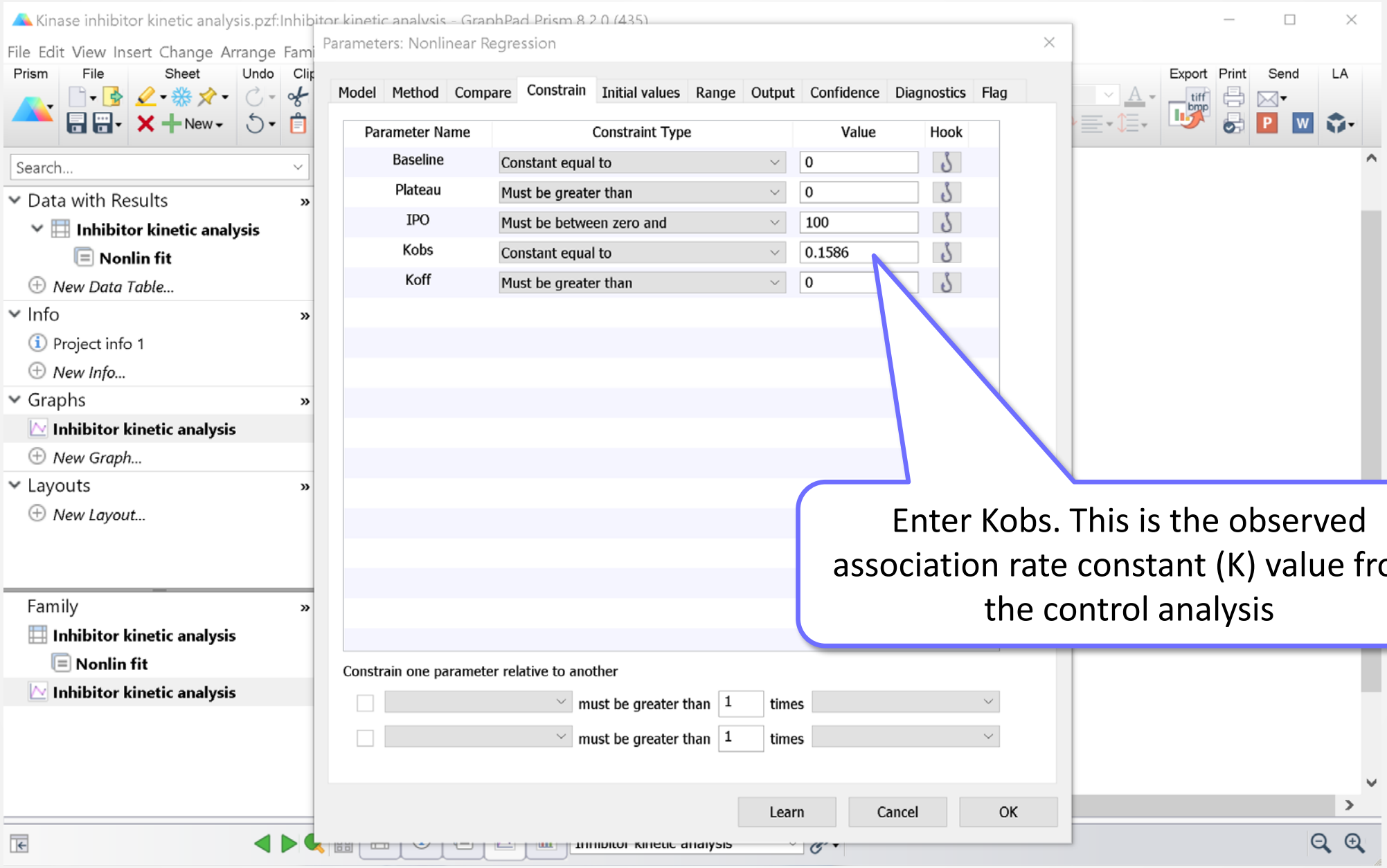
OK
Click “Constrain” tab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Plateau
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IPO
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100
Kobs
Constant equal to
0.1586
Koff
Must be greater than
0
Constrain one parameter relative to another
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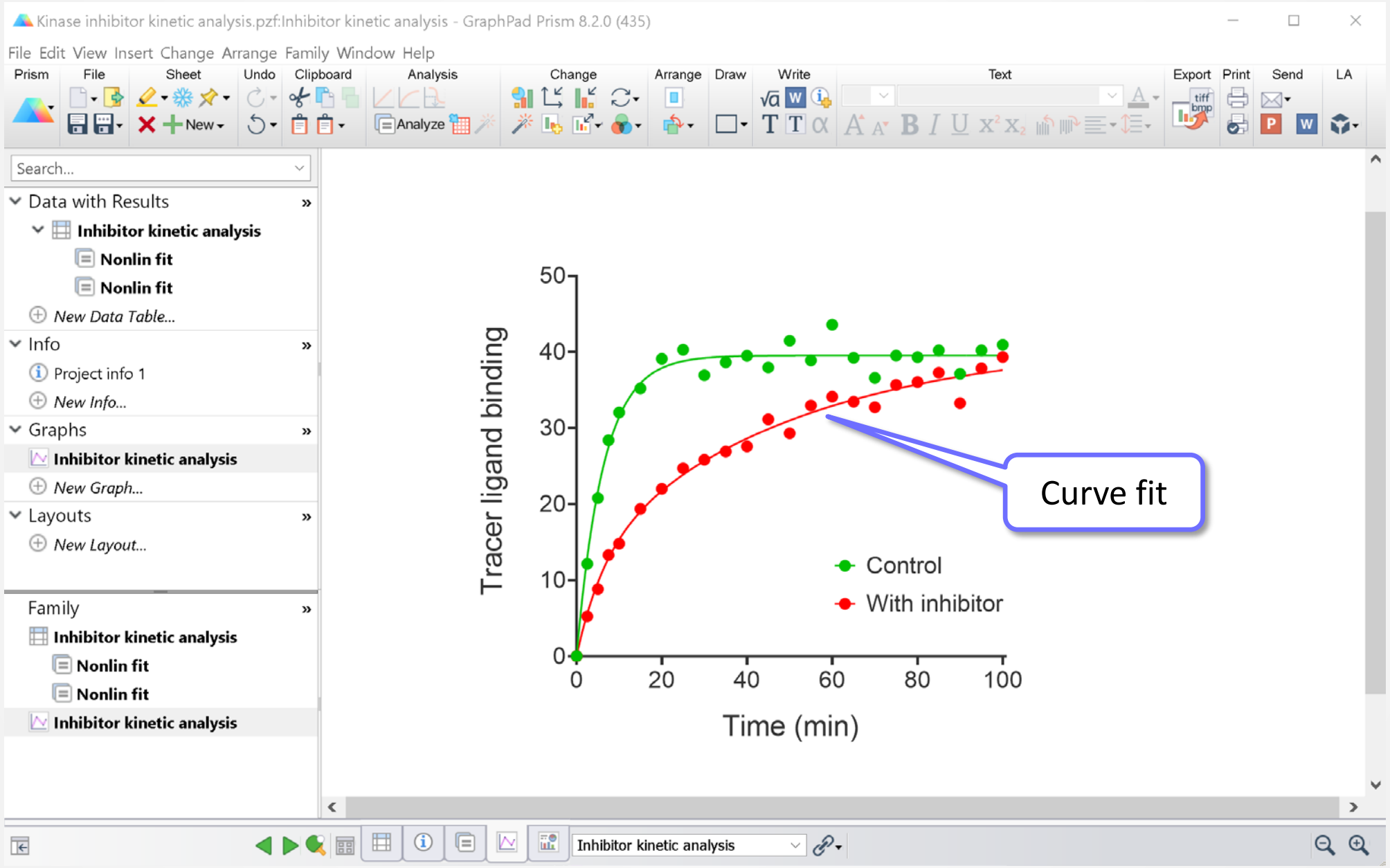
OK
Enter Kobs. This is the observed association rate constant (K) value from the control analysis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Inhibitor kinetic analysis
Tracer ligand binding
50
40
30
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10
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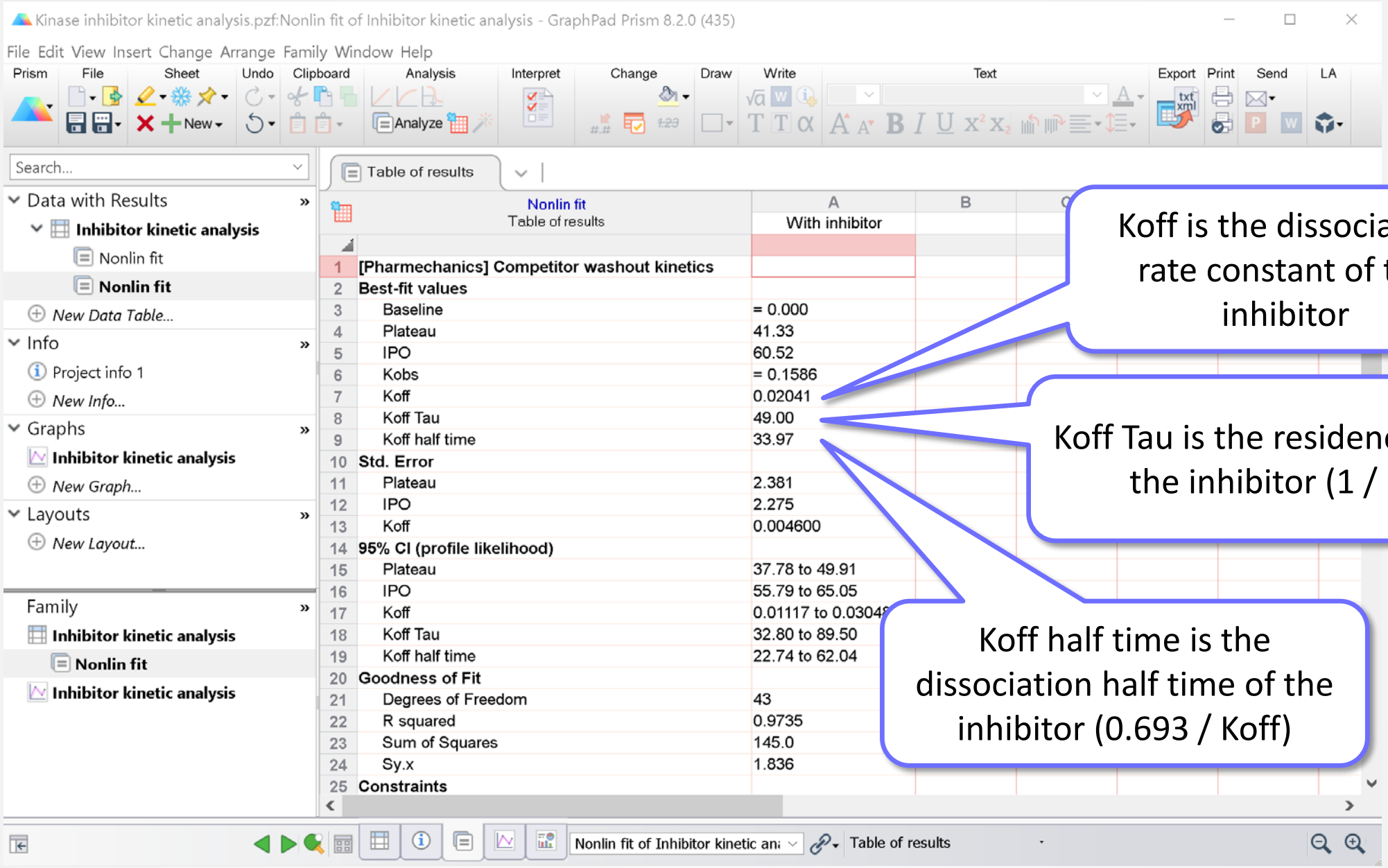
40
60
80
100
Time (min)
Control
With inhibitor
Curve fit
Inhibitor kinetic analysis


Kinase inhibitor kinetic analysis.pzf:Nonlin fit of Inhibitor kinetic analysis - GraphPad Prism 8.2.0 (435)
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Inhibitor kinetic analysis
Nonlin fit
Inhibitor kinetic analysis
Table of results
Nonlin fit
Table of results
A
With inhibitor
B
1 [Pharmechanics] Competitor washout kinetics
2 Best-fit values
3 Baseline = 0.000
4 Plateau 41.33
5 IPO 60.52
6 Kobs = 0.1586
7 Koff 0.02041
8 Koff Tau 49.00
9 Koff half time 33.97
10 Std. Error
11 Plateau 2.381
12 IPO 2.275
13 Koff 0.004600
14 95% CI (profile likelihood)
15 Plateau 37.78 to 49.91
16 IPO 55.79 to 65.05
17 Koff 0.01117 to 0.0304
18 Koff Tau 32.80 to 89.50
19 Koff half time 22.74 to 62.04
20 Goodness of Fit
21 Degrees of Freedom 43
22 R squared 0.9735
23 Sum of Squares 145.0
24 Sy.x 1.836
25 Constraints
Nonlin fit of Inhibitor kinetic ana
Table of results
Koff is the dissociation rate constant of the inhibitor
Koff Tau is the residence time of the inhibitor (1 / Koff)
Koff half time is the dissociation half time of the inhibitor (0.693 / Koff)

Summary and further information

- **An equation has been loaded into Prism for analyzing inhibitor washout kinetics experiments commonly used for kinase targets.**

Equation derivation

Equation derivation

Washout kinetics examples

Methods Mol Biol 2019, 1888: 45-71 (Figs 8 & 13)

Promega webinar (slides 20 & 21)

Aurelia Bioscience examples

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