One Site – Specific Binding

The objective of this case study is to investigate how specific radioligand binding changes as ligand concentration increases and to quantify that relationship using nonlinear curve fitting in **Isalos Analytics Platform**. In this case study, the **One Site – Specific Binding** model is used to estimate the maximum specific binding capacity and the equilibrium dissociation constant using a standard one-site saturation binding framework.

The dataset used in this case study is a published saturation binding dataset describing the binding of **[^3H]-(+)-pentazocine** to **sigma-1 receptors** in **guinea pig liver membranes**, published by Chu *et al.* in Current Protocols in Pharmacology(2015).

It contains measured radioligand concentration values and the corresponding **specific binding** values. In this model, the independent variable (X) is the **radioligand concentration (nM)**, and the dependent variable (Y) is the **specific binding (pmol/mg protein)**. The **One Site – Specific Binding** model describes a saturable hyperbolic relationship in which specific binding increases with radioligand concentration and approaches a limiting maximum at higher ligand levels. The equation used in this analysis is:

$$\boldsymbol{Y = \frac{Bmax \times X}{Kd + X}}$$

In this model, **Bmax** represents the maximum specific binding in the same units as Y, and **Kd** represents the radioligand concentration required to achieve half-maximal binding at equilibrium.

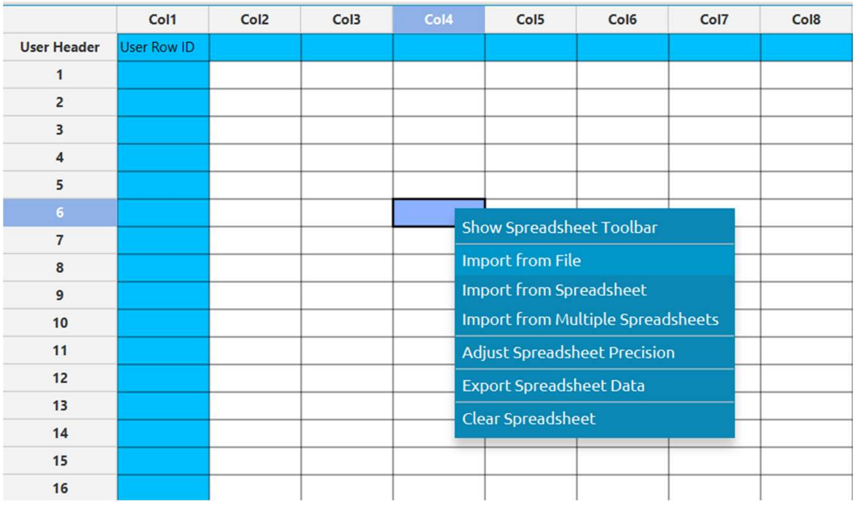
The purpose of this analysis is to determine whether the observed data are consistent with a one-site binding mechanism and to convert the measured binding values into biologically meaningful quantitative outputs. The main results obtained from the fit are **Bmax** and **Kd**, where **Bmax** reflects the total available binding capacity of sigma-1 receptors in the membrane preparation and **Kd** reflects the apparent affinity of the radioligand for the binding site. These results are useful because they allow direct comparison between receptor preparations, ligands, tissues, or assay conditions, supporting interpretation of receptor density and binding affinity.

Isalos version used: 2.0.2

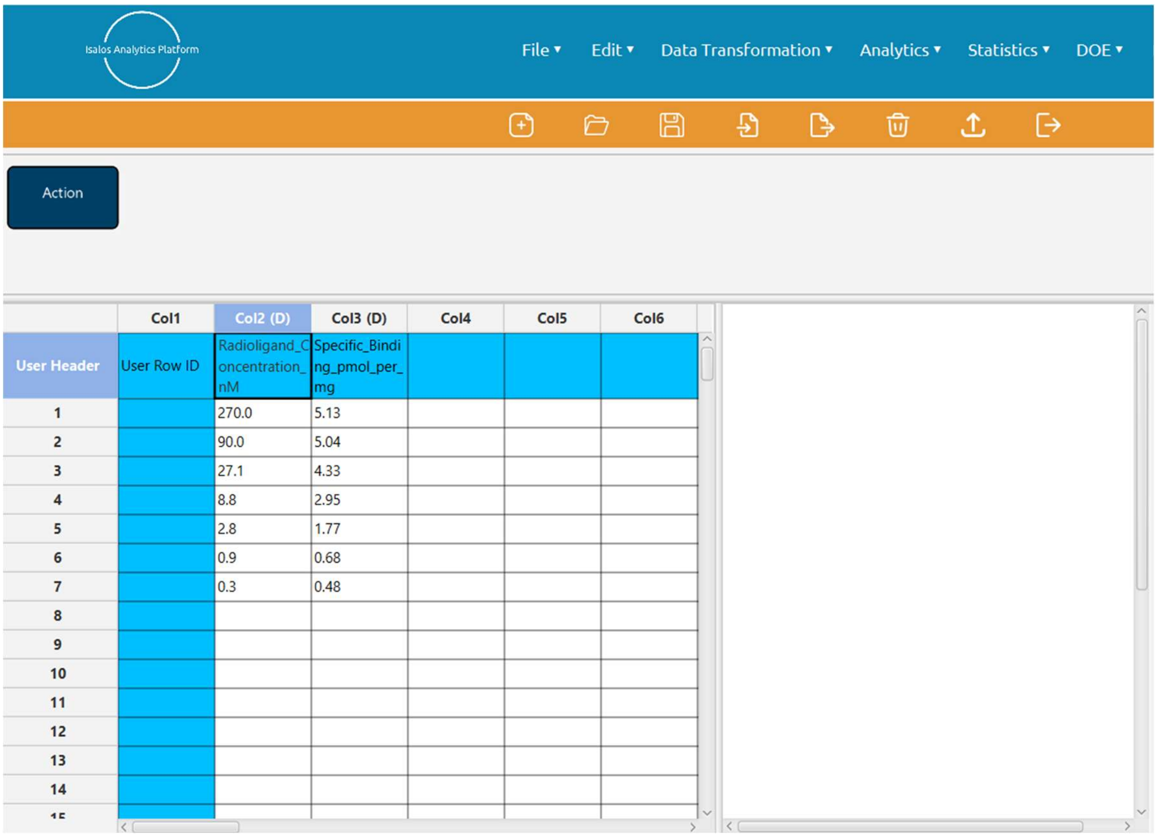
Scientific Article:
https://currentprotocols.onlinelibrary.wiley.com/doi/10.1002/0471141755.ph0134s71

Step 1: Import data from file

Right click on the input spreadsheet (left) and choose the option "**Import from File**". Then navigate through your files to load the one with the receptor-binding data.



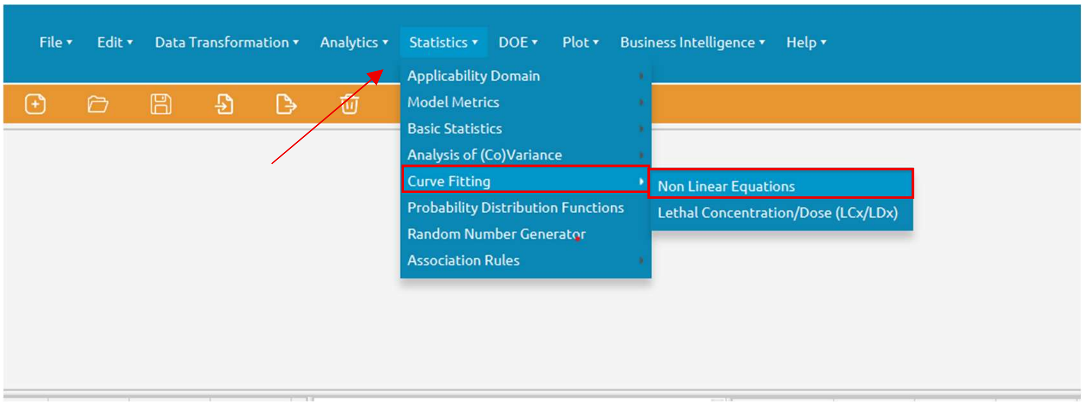
The data will appear on the left spreadsheet.



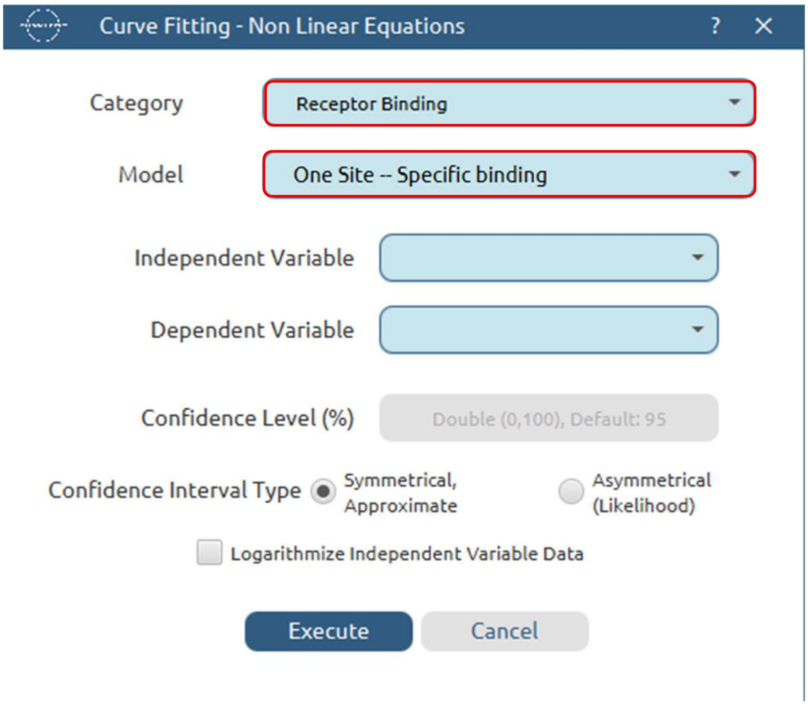
	Col1	Col2 (D)	Col3 (D)	Col4	Col5	Col6
User Header	User Row ID	Radioligand_Concentration_nM	Specific_Binding_pmol_per_mg			
1		270.0	5.13			
2		90.0	5.04			
3		27.1	4.33			
4		8.8	2.95			
5		2.8	1.77			
6		0.9	0.68			
7		0.3	0.48			
8						
9						
10						
11						
12						
13						
14						

Step 2: Select the Statistic Analysis Model

From the toolbar, click on the **Statistics** drop down list, and then choose the desired model and Equation category navigating through *Statistics > Curve Fitting > Non Linear Equations.*



In the category list, select **Receptor Binding** and then choose the **One Site – Specific Binding** model on the model menu.



Category: Receptor Binding

Model: One Site -- Specific binding

Independent Variable:

Dependent Variable:

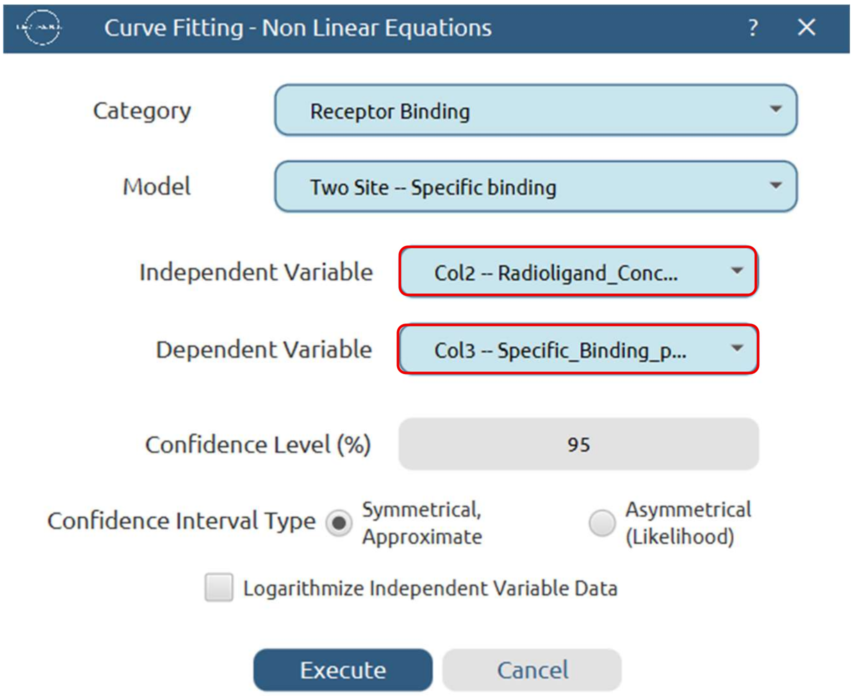
Confidence Level (%): Double (0,100), Default: 95

Confidence Interval Type ● Symmetrical, Approximate ○ Asymmetrical (Likelihood)

☐ Logarithmize Independent Variable Data

Step 3: Configure Variables and Confidence Intervals

Set **"Radioligand_Concentration"** as the independent variable X and **"Specific_Binding_fmol_per_mg"** as the dependent variable Y, using the dataset provided for this case study. Set the confidence level to **95%** and choose **Symmetrical Approximate** as the confidence interval type.



Curve Fitting - Non Linear Equations

Category: Receptor Binding

Model: Two Site -- Specific binding

Independent Variable: Col2 -- Radioligand_Conc...

Dependent Variable: Col3 -- Specific_Binding_p...

Confidence Level (%): 95

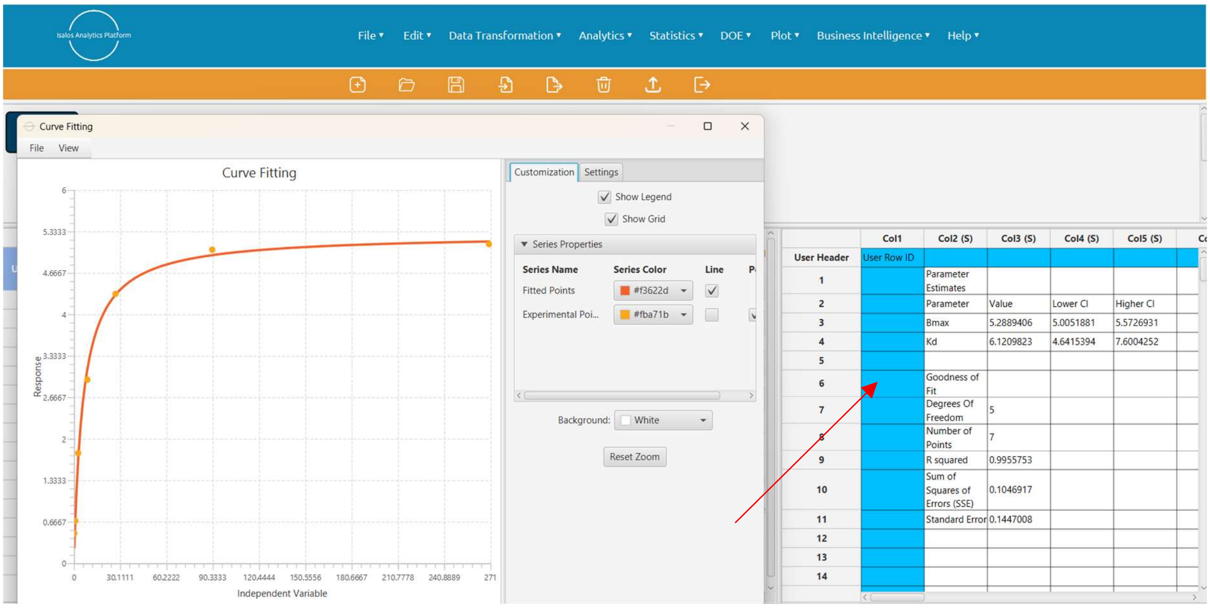
Confidence Interval Type: ● Symmetrical, Approximate ○ Asymmetrical (Likelihood)

☐ Logarithmize Independent Variable Data

Step 4: Analyzing the Output and Fitted Curves

Once the analysis is completed, Isalos displays the fitted curve with the experimental data points, to visually assess how well the **One Site – Specific Binding** model describes the observed saturation pattern. In the output table(right), you can review the **Parameter Estimates**, which report the fitted values of the model parameters together with their lower and upper confidence limits at the selected confidence level. For this model, the main parameters are **(Bmax),** representing the maximum specific binding, and **(Kd),** representing the ligand concentration required to achieve half-maximal specific binding at equilibrium.

The **Goodness of Fit** section of the table, summarizes key fitting statistics such as the **number of data points used**, **degrees of freedom**, **residual sum of squares**, and the **standard error** of the regression. These outputs should be considered together with the fitted plot to evaluate how well the model describes the data and how reliable the estimated parameters are.



☑ Show Legend
☑ Show Grid

Series Properties

Series Name	Series Color	Line
Fitted Points	🟥 #f3622d	☑
Experimental Poi...	🟧 #fba71b	☐

Background: White

	Col1	Col2 (S)	Col3 (S)	Col4 (S)	Col5 (S)
User Header	User Row ID				
1		Parameter Estimates			
2		Parameter	Value	Lower CI	Higher CI
3		Bmax	5.2889406	5.0051881	5.5726931
4		Kd	6.1209823	4.6415394	7.6004252
5					
6		Goodness of Fit			
7		Degrees Of Freedom	5		
8		Number of Points	7		
9		R squared	0.9955753		
10		Sum of Squares of Errors (SSE)	0.1046917		
11		Standard Error	0.1447008		
12					
13					
14					

The fitted results indicate that the binding of **[^3H]-(+)-pentazocine to sigma-1 receptors** in guinea pig liver membranes is well described by a one-site saturation binding model. A **Bmax** value of approximately **5.29 pmol/mg** suggests that the membrane preparation has a finite and measurable binding capacity, corresponding to the total available sigma-1 receptor binding sites detected under these assay conditions. A **Kd** value of approximately **6.12 nM** indicates that half-maximal specific binding is achieved at a relatively low nanomolar radioligand concentration, which is consistent with appreciable binding affinity in this preparation.

The narrow confidence intervals for both parameters, with the high $\boldsymbol{R}^2$ value and low residual error, suggest that the model provides a good description of the observed saturation binding data and that the estimated **Bmax** and **Kd** values are reliable.

References:

(1) Chu, U.B. and Ruoho, A.E., 2015. Sigma receptor binding assays. *Current protocols in pharmacology*, *71*(1), pp.1-34.