

# Binding Tutor

The logo for Binding Tutor features the word "Binding" in a dark green, sans-serif font, with the letter "g" partially overlapping a large, light green circle. Below "Binding", the word "Tutor" is written in a lighter green, sans-serif font. A small, dark green circle is positioned above the "g" in "Binding".

**Version 0.1**

Julia Philip,<sup>1</sup> Charles Pence,<sup>2</sup> Holly Goodson<sup>1</sup>

BindingTutor is a program to plot binding curves under various conditions.  
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<sup>1</sup>University of Notre Dame, Department of Chemistry and Biochemistry

<sup>2</sup>University of Notre Dame, Program in History and Philosophy of Science

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# 1. GETTING STARTED

Thanks for checking out BindingTutor! Here's a quick-start tutorial designed to get you graphing binding curves in no time.

## 1.1. Running BindingTutor for the First Time

To begin, you will need to [download and install BindingTutor](#). When you run BindingTutor for the first time, you should see a window that looks like this:

The screenshot shows the MTBindingSim software interface. The window title is "MTBindingSim".

**Experimental Method:**

- Vary [MT]
- Vary [A]
- Competition

**Plotting Mode:**

- Plot a single curve
- Compare two curves

**X-axis:**

- [MT] free
- [MT] total

**Number of points:** 100

**[MT] total min:** 0  $\mu\text{M}$

**[MT] total max:** 0  $\mu\text{M}$

**Buttons:** Save Graph, Graph Curve

**Binding Type:** First order binding (with a help icon)

**Chemical Equation:**  $A + MT \leftrightarrow AMT$

**Equation:**  $K_{AMT} = [A][MT]/[AMT]$

**Parameters:**

- [A] total: 0  $\mu\text{M}$
- $K_{AMT}$ : 0  $\mu\text{M}$
- 1 MT: 1 A

BindingTutor's defaults are to plot a graph of an experiment in which the total concentration of ligand, [L], is varied (along the X-axis), and the binding model is first-order binding. See [the Running BindingTutor chapter](#) for more information about these settings, and the [Binding Model Mathematics chapter](#) for more detailed information about the binding models.

First, enter values for the experimental range of [L]. Varying between 0 and 10 is a nice start.

[MT] total min	<input type="text" value="0"/>	$\mu\text{M}$
[MT] total max	<input type="text" value="10"/>	$\mu\text{M}$

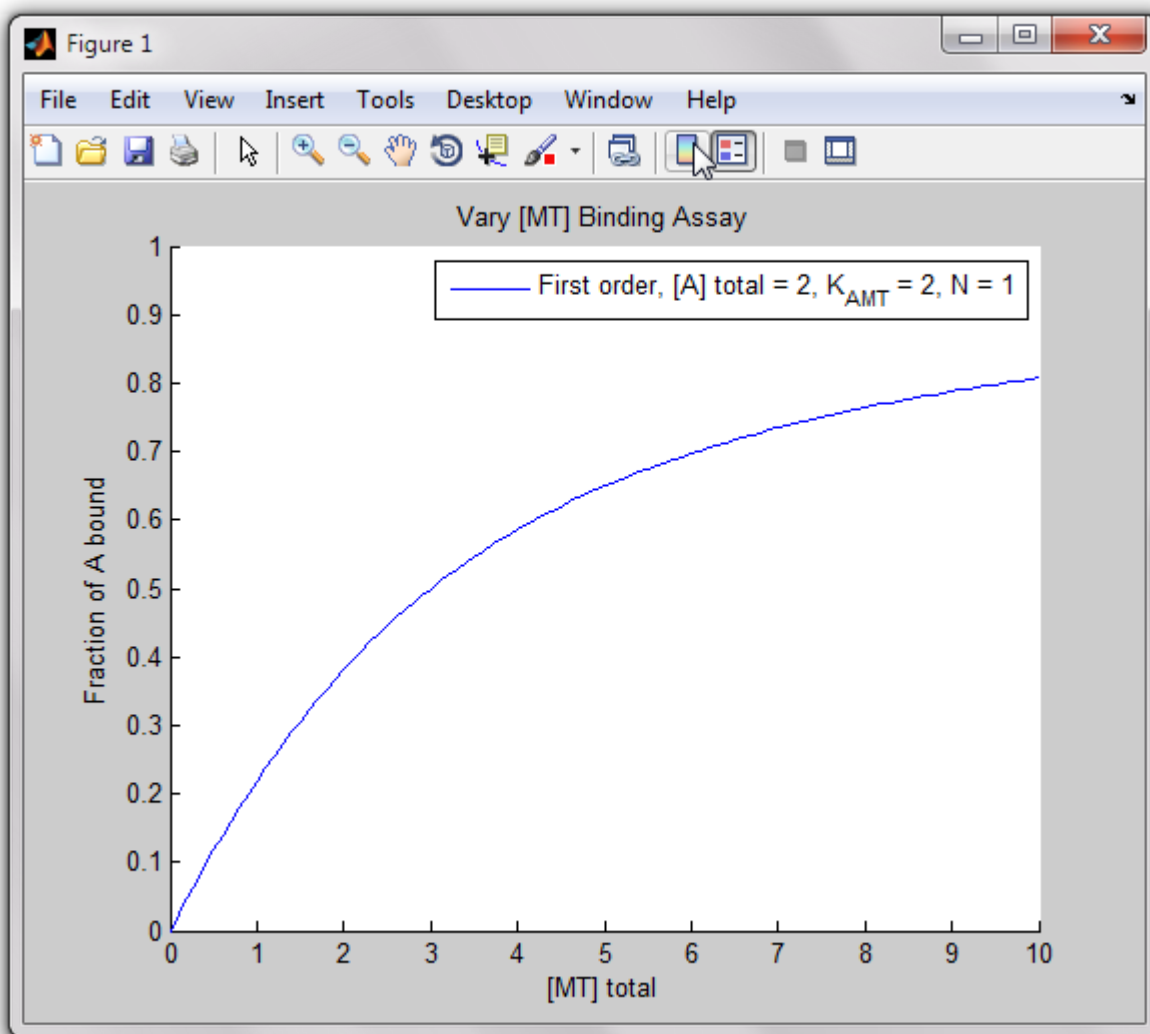
You will also need to enter values for [P] total and  $K_D$ . Try 2 for both [P] total and  $K_D$ .

[A] total	<input type="text" value="2"/>	$\mu\text{M}$
$K_{AMT}$	<input type="text" value="2"/>	$\mu\text{M}$
1 MT :	<input type="text" value="1"/>	A

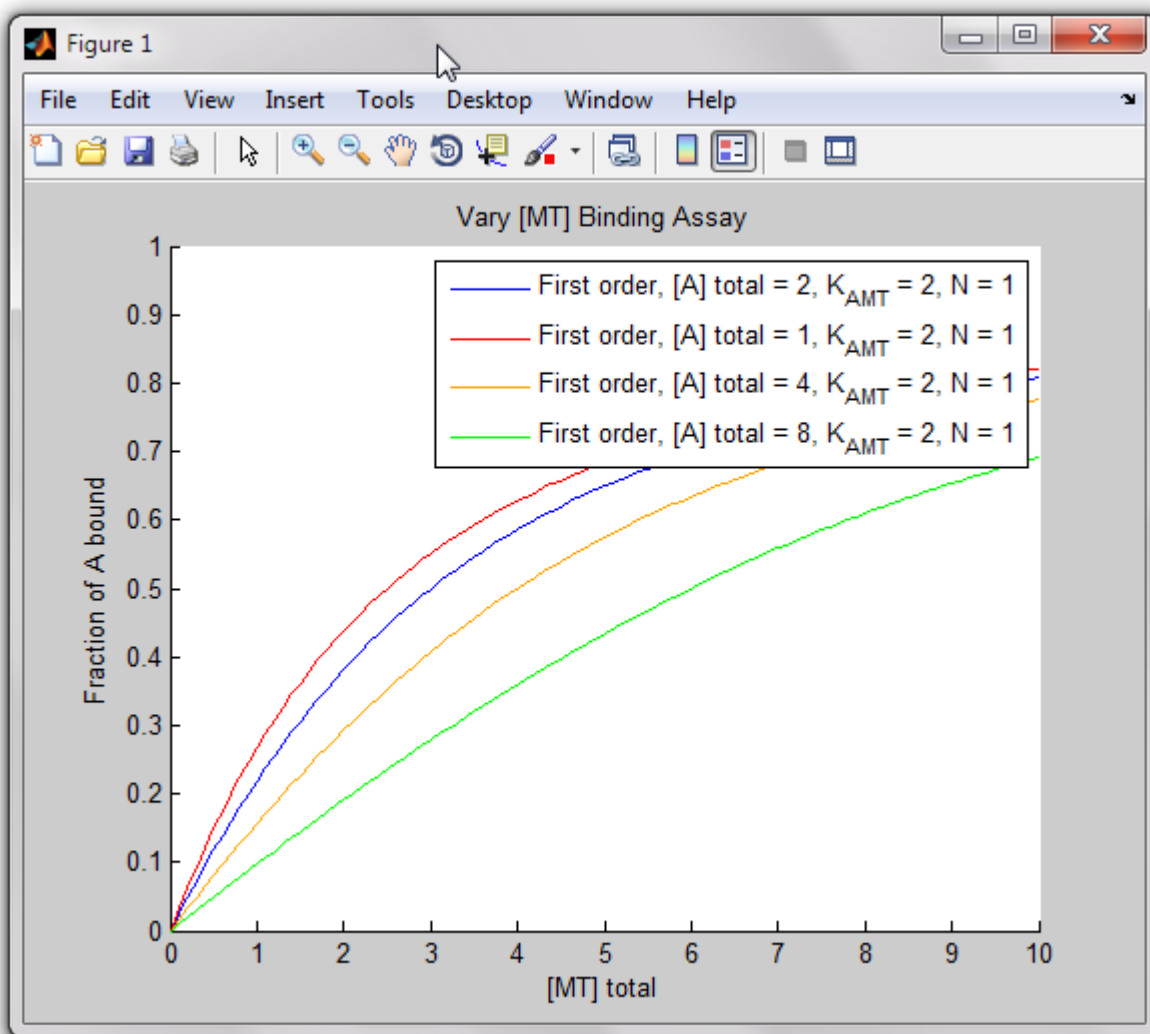
To calculate the binding curve under these conditions, click the graph button.

<input type="button" value="Graph Curve"/>
--

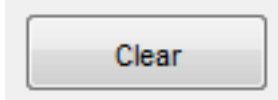
You should now see a graph that looks like this:



Congratulations, you've just plotted your first binding curve using BindingTutor!  
Try changing the values of [P] total or  $K_D$  to see how the curves change. After plotting four curves you'll have a graph that might look something like this:



If you'd like to start over with a new graph, click the clear button. This will delete the current graph, and subsequent curves will be plotted on a new plot.



For more detailed information about what you can do with BindingTutor, see the [Running BindingTutor](#) and [How To...](#) chapters. If you have any trouble, see the [Troubleshooting](#) section.

Good luck, and happy graphing!

## 2. RUNNING BINDING TUTOR

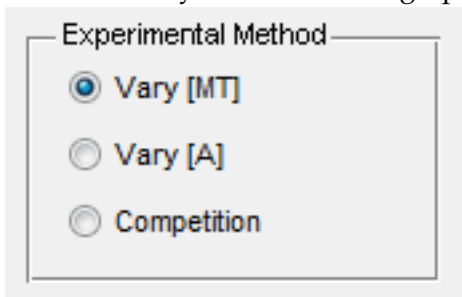
To plot a graph using BindingTutor, select the plotting options and binding model you would like to use, enter the parameters you want, and click the graph button. This section explains what plotting options and binding models are available.

### 2.1. Plotting Options

There are several sets of plotting options that can be selected. These options will apply to all graphed curves.

#### 2.1.1. Experimental Method

There are three available experimental methods. Curves from different experimental methods cannot be plotted on the same graph, so changing the experimental method will automatically close an active graph.



**Vary [L]:** In this method, the concentration of P is held constant and the concentration of L is varied. The fraction of P bound is graphed on the y-axis. This is a “standard” binding experiment and simulates data from many standard experimental methods of measuring binding data, including SPR, fluorescence anisotropy, and tryptophan fluorescence, as well as cosedimentation assays.

**Vary [P]:** In this method, the concentration of L is held constant and the concentration of P is varied. The concentration of P bound is graphed on the y-axis. This kind of data cannot be collected using many standard binding assays such as SPR and fluorescence anisotropy, but it can be collected using a cosedimentation assay.

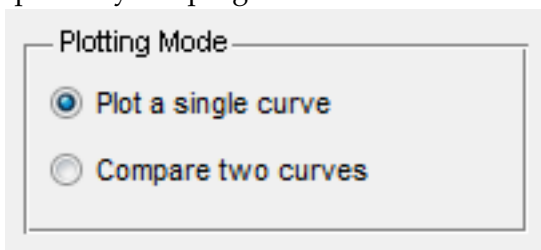
**Competition:** In this method, there are two L binding proteins, P and B. The concentration of P and the concentration of L is held constant while the concentration of B is varied. The fraction of P bound is plotted on the y-axis. This kind of data can be collected with many standard methods of measuring binding data, such as SPR, fluorescence anisotropy, tryptophan fluorescence, and cosedimentation assays.

#### 2.1.2. Plotting Mode

BindingTutor can plot one curve at a time or it can plot two curves simultaneously and compare them. You may plot as many curves as you wish as long as you don't change



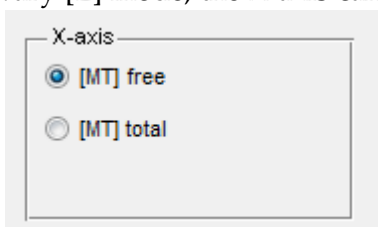
the experimental method or X-axis settings. However, only two curves may be directly compared by the program.



For more information about comparing two curves see the [How To](#) section.

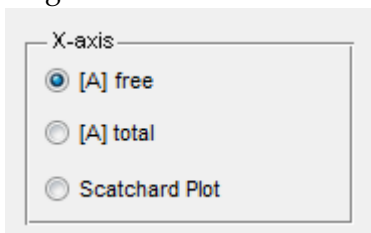
### 2.1.3. X-axis

In Vary [L] mode, the X-axis can be set to either [L] total or [L] free.



[L] total is a known quantity in the experiment, while [L] free must be calculated. However, the familiar Langmuir Isotherm equation uses [L] free as its independent variable, and the quick method of determining  $K_D$  by looking at the L concentration when the fraction of P bound is 0.5 *only* works if the x-axis is [L] free. In either X-axis mode the Y-axis will be the fraction of P bound.

In Vary [P] mode there are three possible kinds of plots. In the first two the Y-axis is the concentration of P bound to L, and the X-axis can be set to [P] free or [P] total. In addition, the data can be plotted in a Scatchard plot, where the X-axis is [P] bound and the Y-axis is [P] bound/[P] free. This kind of plot is used to make the binding data linear. Binding data will be linear for simple interactions and a curved line in a Scatchard plot indicates the presence of cooperativity or other kinds of non-simple binding interactions.



Changing the x-axis mode will automatically close the active graph.

### 2.1.4. Number of Points

The number of points plotted may be specified.

Number of points

The default number of points is 100. A larger number of points can be used if a smoother curve is desired. A smaller number of points will result in a faster calculation.

## 2.2. Binding Models

BindingTutor can plot curves for several binding models. For a detailed description of the math used to generate the curves, please see the [Binding Model Mathematics chapter](#). Note that all models use the dissociation constant,  $K_D$ .

The info button to the right of the model selection box provides a brief description of each model, reproduced below.

First order binding

First order binding: Simple P binds L interaction. This model is valid for any simple protein-protein or protein-ligand interaction.

Two binding sites: P can bind to two sites on each L. This model is valid for any protein-protein or protein-ligand interaction with two independent binding sites.

### 2.2.1. First Order Binding

First order binding simulates binding under standard first order conditions where one P interacts with one L, and all P-L interactions are identical.

First order binding

$A + MT \leftrightarrow AMT$

$K_{AMT} = [A][MT]/[AMT]$

[A] total   $\mu\text{M}$

$K_{AMT}$    $\mu\text{M}$

1 MT:  A

For first order binding, you need to input the total concentration of P (or L) and the  $K_D$ . All of these must be positive numbers.

### 2.2.2. Two Binding Sites

In this model, P can bind to two sites per ligand, site 1 and site 2 with different dissociation constants.

2 Sites

$A + MT1 \leftrightarrow AMT1, A + MT2 \leftrightarrow AMT2$

$K_{AMT1} = [A][MT1]/[AMT1], K_{AMT2} = [A][MT2]/[AMT2]$

[A] total   $\mu\text{M}$

$K_{AMT1}$    $\mu\text{M}$

$K_{AMT2}$    $\mu\text{M}$

In this model, you input the total amount of P (or L), the dissociation constant for P binding to  $L_1$  sites,  $K_{D1}$  and the dissociation constant for P binding to  $L_2$  sites,  $K_{D2}$ . All inputs must be positive numbers. Note that the total amount of L is the total amount of  $L_1$  and  $L_2$ .

This model cannot be graphed with an x-axis of [L] free.

This chapter explains how to use some of the advanced features of BindingTutor.

### 3.1. Editing and Saving Graphs

#### 3.1.1. Saving Graphs

To save a graph generated by BindingTutor for later use or editing click on the save graph button. You can save your figure in the following formats:

- MATLAB figure (.fig)
- Adobe Illustrator file (.ai)
- EPS file (.eps)
- JPEG image (.jpg)
- Portable Document Format (.pdf)
- Portable Network Graphics file (.png)
- TIFF image (.tif)
- Excel spreadsheet (.xls)
- Comma separated value table (.csv)

If you wish to embed your figure in a PowerPoint presentation or a Word document, the best choice is probably a JPEG image. If you wish to include your figure in a LaTeX document, you should probably save it either as a PDF file or as an EPS file. Finally, if you wish to edit the graph, changing text or other graph properties, it is recommended to save the graph either in Adobe Illustrator format (if you have access to Illustrator), or in PNG format (for editing in any image editing software, including Photoshop).

If you'd like to save the curves as x and y values in a spreadsheet, see "Saving a Graph as a Spreadsheet" below.

#### 3.1.2. Editing Graphs

The graphs generated by BindingTutor can be edited in several ways. From inside the program, you can change the location of the legend box by clicking and dragging. You also can edit the legend text by double-clicking on it.

If you would like to see a particular part of the graph blown up, you can use the zoom in and out tools on the graph window, and then the hand tool to drag the graph to the desired region.

To edit the graph more extensively, two options are available. If you have MATLAB, you can save the graph as a MATLAB Figure (.FIG) file, and edit it as you would any other MATLAB graph. Otherwise, it is recommended to save as an Adobe Illustrator

(.AI) file, and edit in Adobe Illustrator. You may also be able to edit an Illustrator file in the open-source [Inkscape](#) image editor.

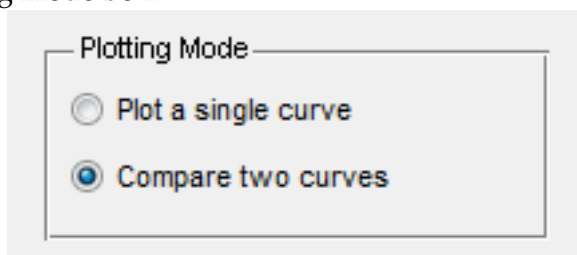
### 3.2. Saving a Graph as a Spreadsheet

To save the curves from a graph as x and y values in a spreadsheet, click on the save graph button in BindingTutor. Saving as an Excel file (.xls) will generate an Excel file containing the x and y values for all curves on the graph. Saving as a comma separated value file (.csv) will create a CSV table with the x and y values for all curves on the graph.

If you are running BindingTutor in Mac OS X or Linux, you cannot save your files as .xls. You can save them as .csv files and then open them in Excel. Please see [Troubleshooting](#) for more information.

### 3.3. Comparing Two Curves

To compare two curves in BindingTutor, first select “compare two curves” from the plotting mode box.



This will cause a second model selection drop down box and set of model parameter input boxes to appear.

Enter the parameters for the curves you wish to compare in the two columns. The curves are independent of each other. An example of two curves to be compared is below.

MTBindingSim

**Experimental Method**

Vary [MT]

Vary [A]

Competition

**Plotting Mode**

Plot a single curve

Compare two curves

**X-axis**

[MT] free

[MT] total

Number of points

[MT] total min   $\mu\text{M}$

[MT] total max   $\mu\text{M}$

First order binding   $A + MT \leftrightarrow AMT$

$K_{AMT} = [A][MT]/[AMT]$

[A] total   $\mu\text{M}$

$K_{AMT}$    $\mu\text{M}$

1 MT :  A

MT seam and lattice binding  $A + S \leftrightarrow AS, A + L \leftrightarrow AL$

$K_{AL} = [A][L]/[AL], K_{AS} = [A][S]/[AS]$

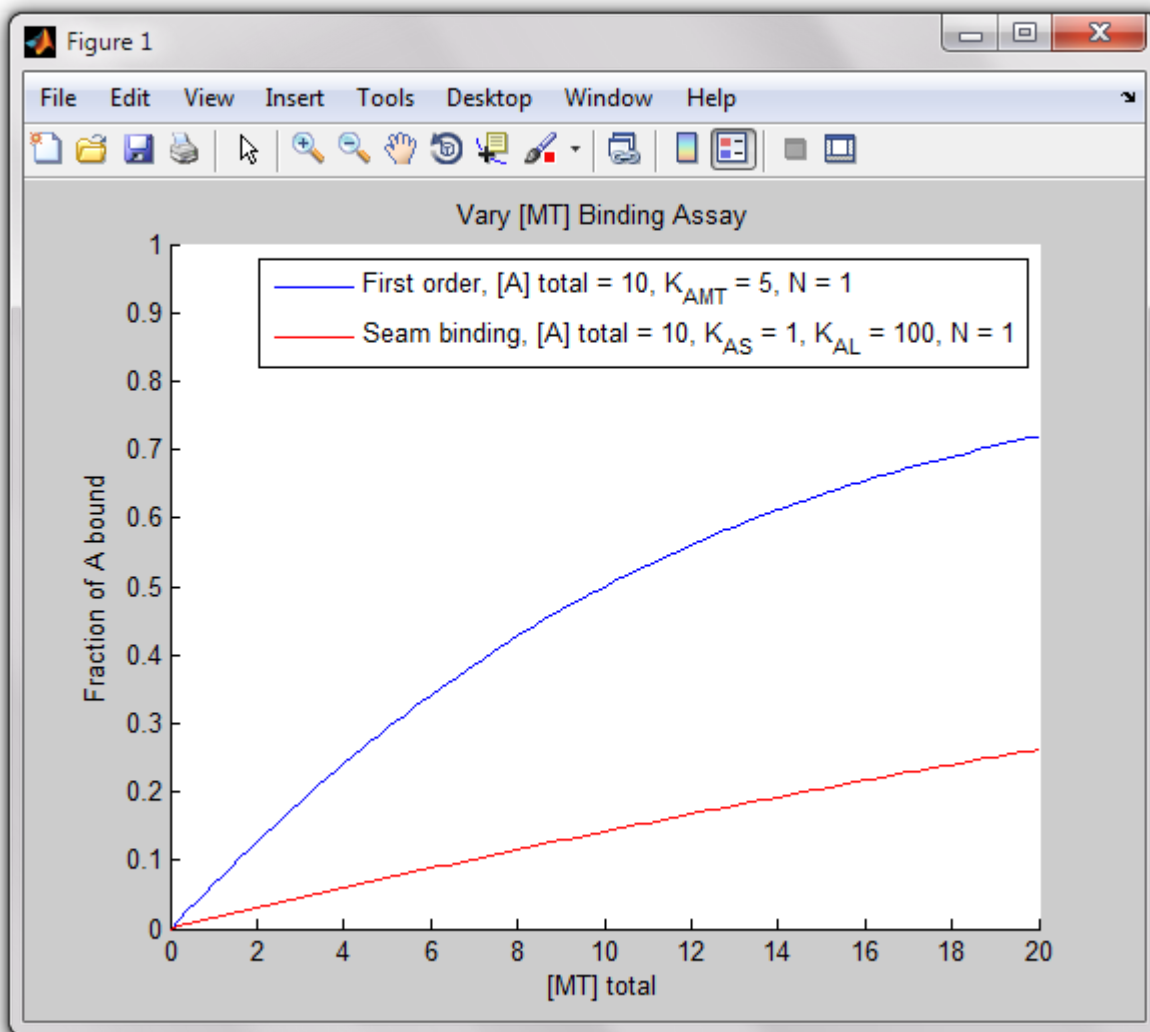
[A] total   $\mu\text{M}$

$K_{AS}$    $\mu\text{M}$

$K_{AL}$    $\mu\text{M}$

1 MT :  A

When you click the graph button, the curves will be plotted on the active graph or a new graph.



If the X-axis is plotting total [L] or total [P], or in competition mode the program also will calculate the difference between the two curves and display the result.

Average absolute difference: 0.31302  
 Average percent difference: 109.485%  
 Maximum absolute difference is 0.45691 at 20 [MT] total  
 Maximum percent difference is 124.8279% at 0.2 [MT] total

These values are calculated by computing the absolute and percentage difference between the curves at each point, and both the average and largest difference are reported for each. This feature is designed to help researchers determine whether the predicted differences between two curves are detectable given their expected experimental error.

This comparison is only done for curves plotted with the X-axis as [L] total or [P] total because the x-values for both curves are the same in that case, so the comparison calculations are unambiguous. When the X-axis is [L] free or [P] free the x-values for the curves will be different and the calculation of a comparison between the curves requires making assumptions about the binding behavior.

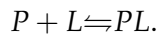
## 4. BINDING MODEL MATHEMATICS

This chapter describes the equations used to simulate the binding curves. Though some of these equations can be solved analytically, the calculations are performed numerically. The program breaks either the total [P] or total [L] range into a user-defined number of points and then calculates the concentration of free and bound A and free and bound MT at each point.

### 4.1. First Order Binding

This model is simple first order binding.

In first order binding, the relationship between P and L is:



The dissociation constant is defined as:

$$K_D = \frac{[P][L]}{[PL]}.$$

We can also write mass balances for total P and total L:

$$[P]_{\text{total}} = [P] + [PL] = [P] + \frac{1}{K_D}[P][L]$$

$$[L]_{\text{total}} = [L] + [PL] = [L] + \frac{1}{K_D}[P][L] = [L](1 + \frac{1}{K_D}[P]).$$

We can rearrange the equation for total L and solve for [L] free:

$$[L] = \frac{[L]_{\text{total}}}{1 + \frac{1}{K_D}[P]}.$$

We now can substitute this equation into the equation for total P:

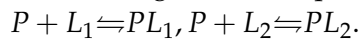
$$[P]_{\text{total}} = [P] + \frac{\frac{1}{K_D}[P][L]_{\text{total}}}{1 + \frac{1}{K_D}[P]}.$$

The program numerically finds the value of [P] free that solves this equation, then uses that to calculate all other necessary parameters.

### 4.2. Two Binding Sites

This model assumes that each ligand contains two binding sites for protein P, sites 1 and 2, with different dissociation constants. It is assumed that the two sites do not interact.

The binding relationships for this model are:



The dissociation constants for this model are:

$$K_{D1} = [P][L_1]/[PL_1], K_{D2} = [P][L_2]/[PL_2].$$

The mass balances for this model are:

$$[P]_{\text{total}} = [P] + [PL_1] + [PL_2] = [P] + [P][L_1]/K_{D1} + [P][L_2]/K_{D2} = [P](1 + [L_1]/K_{D1} + [P][L_2]/K_{D2}),$$

$$[L_1]_{\text{total}} = [L_1] + [PL_1] = [L_1] + [P][L_1]/K_{D1} = [L_1](1 + [P]/K_{D1}),$$

$$[L_2]_{\text{total}} = [L_2] + [PL_2] = [L_2] + [P][L_2]/K_{D2} = [L_2](1 + [P]/K_{D2}).$$

The  $L_1$  and  $L_2$  mass balances can be solved for free  $L_1$  and  $L_2$ :

$$[L_1] = \frac{[L_1]_{\text{total}}}{1 + [P]/K_{D1}},$$



$$[L_2] = \frac{[L_2]_{\text{total}}}{1 + [P]/K_{D2}}.$$

These equations can be substituted into the mass balance for P to get:

$$[P]_{\text{total}} = [P] + \frac{[P][L_1]_{\text{total}}}{K_{D1}(1 + [P]/K_{D1})} + \frac{[P][L_2]_{\text{total}}}{K_{D2}(1 + [P]/K_{D2})}.$$

This equation is numerically solved by the program to get free P, which is then used to calculate bound P and the fraction of P bound. Free L is not calculated because this model cannot be graphed against free L.

## 5. TROUBLESHOOTING

### 5.1. Downloading and Installation

#### 5.1.1. There are password-protected files in your ZIP file

or

#### 5.1.2. I can't open your ZIP file

If you are running Windows, there is a bug in the built-in Windows ZIP file extractor that occasionally causes trouble with our releases. Try downloading and installing the free and open source [7-Zip ZIP extractor](#), and extracting BindingTutor using it instead. If you are not running Windows, or if you have trouble even when using 7-Zip, please file a support ticket (see our specific information about [Filing a Support Ticket](#) below).

#### 5.1.3. BindingTutor doesn't run

On a Mac, make sure you have downloaded and installed the MATLAB Compiler Runtime (MCR), available at [the BindingTutor downloads page](#).

On Windows, the installer should download and install the MATLAB Compiler Runtime (MCR) if you don't already have it. If you get an error that the MCR cannot be found, you probably have an old version of the MCR, which prevents the installer from installing the new version. Uninstall the old version if you don't need it for any other programs and then manually download and install the updated version from [the BindingTutor downloads page](#).

If you have successfully installed all the prerequisites and still cannot get BindingTutor to run, try [Filing a Support Ticket](#) below.

### 5.2. Graphing

#### 5.2.1. My graph doesn't show up

If you graph multiple simulations on the same graph, the axes will be set to show the full range of all curves. If you have curves with very different values, you may not be able to see all of the curves on the same graph. Try closing the graph and making a new graph, paying attention to the range of the X- and Y-axis.

Also, if you graph two very nearly identical graphs at the same time, the line for the last one to be graphed may lie precisely on top of the line for the first to be graphed, making the first line invisible. Try graphing your curves one-at-a-time, and comparing the graphs.

## 5.2.2. My graph looks wrong

Your graph looking wrong could have several causes. First, make sure that the x- and y-axis ranges are appropriate for your graph.

If you are confident that you are looking at the graph with appropriate axes and it still looks wrong or strange, it is possible that the program has calculated your graph incorrectly. While we have made every attempt to ensure that BindingTutor will always calculate the correct graph, it is possible that we missed something. Please submit a bug report on our website and we will attempt to fix the problem. To help us diagnose the issue you are having, it would be very helpful if you can run the graph several times, tweaking the parameters, to determine what parameter or combination of parameters is causing the problem. Thank you for helping us keep BindingTutor working properly!

## 5.3. Saving Your Results

### 5.3.1. I can't save XLS files

MATLAB saves XLS-format spreadsheets by communicating with Microsoft Excel in a manner which only works on Windows. If you wish to save a spreadsheet on Mac OS X or Linux, you can save in CSV format instead. CSV files can be opened by any version of Microsoft Excel or your favorite spreadsheet program.

For more information about the limitations of MATLAB's communication with Microsoft Excel, see [MathWorks Solution 1-2SJUON](#).

## 5.4. Other

### 5.4.1. I need help, can I contact the developers?

or

### 5.4.2. I think I've found a bug, how do I report it?

or

### 5.4.3. I have an idea for a great new feature!

Head to the next section and learn about Filing a Support Ticket.

## 5.5. Filing a Support Ticket

BindingTutor is hosted on GitHub, and we use its "Issues" system for keeping track of support requests. For your best chance at support, please file a ticket there, rather than e-mailing one of the developers.

When should you file a support ticket? In general, if you have a question that you just can't get answered in the documentation, if you have a bug to report, or if you have an idea for a new feature, you should send us a message.

Rather than including it in the documentation, we keep up-to-date information about [how to file a support ticket](#) on the BindingTutor website. Please visit there for the latest information about how to get BindingTutor support.

## 6. CONTACT US

If you would like to get in touch with the developers by e-mail, you may do so at the following addresses:

- [Julia Philip](#)
- [Charles Pence](#)

Note that if you are looking for support, to file a bug report, or to request an issue, we would much prefer if you filed an issue in our support system on Google Code. See our [page about creating support tickets](#).

## 7. ACKNOWLEDGEMENTS

Many people have contributed to the process of writing BindingTutor and we would like to thank them.

The lead developers are Julia Philip and Charles Pence. Julia was supported by the Notre Dame Chemistry, Biochemistry, and Biology Interface Program, NIH Training Grant T32GM075762 and NSF grant 0951264.

We would like to thank Dr. Holly Goodson for support and guidance, and Eric Bunker for assistance with writing the documentation and troubleshooting the program.

BindingTutor is an outgrowth of [MTBindingSim](#) designed for use in teaching. We thank Dr. Goodson for allowing us to test the use of MTBindingSim/BindingTutor in her Biochemistry for Engineers class at the University of Notre Dame and Dr. Jill Voreis for running the instructional sessions using MTBindingSim/BindingTutor and designing surveys to test its effectiveness as a teaching tool.

# A. BINDINGTUTOR REVISION HISTORY

## A.1. Version 0.1

- Initial release.