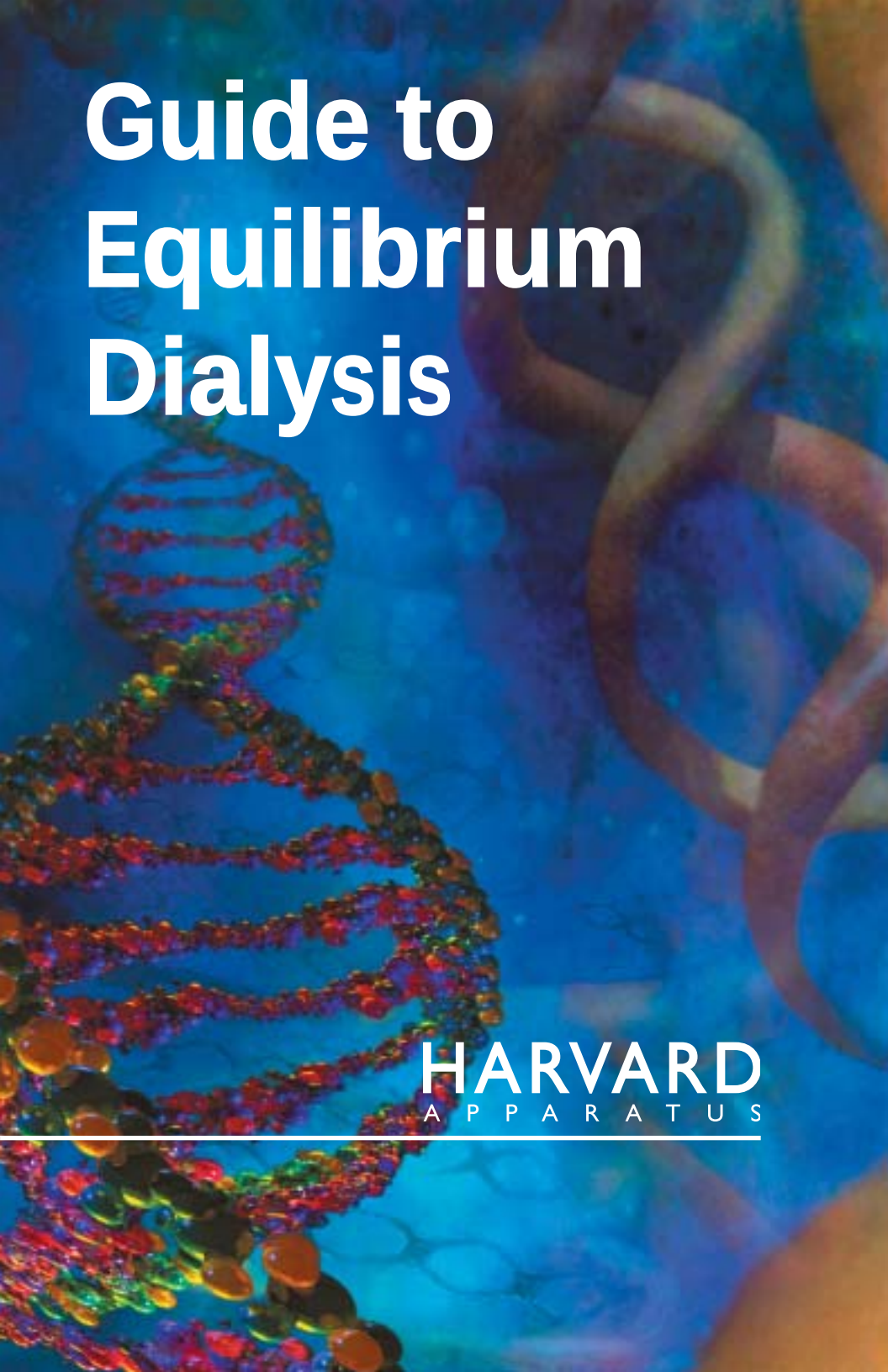
Guide to Equilibrium Dialysis



HARVARD
APPARATUS

Acknowledgement
The sections of this booklet about mass-action and regression were adapted, with permission, from H.J. Motulsky, Analyzing Data with GraphPad Prism, GraphPad Software, 1999. Additional information is available on line at www.curvefit.com.

Distributed by:

The Nest Group, Inc.
45 Valley Road
Southborough, MA 01772-1323

Phone: (800) 347-6378 or 508-481-6223
Fax: (508) 485-5736
Web: www.nestgrp.com
E-mail: info@nestgrp.com

Contents

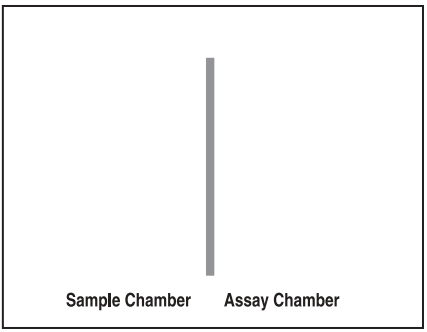
Introduction

Equilibrium Dialysis is a simple but effective tool for the study of interactions between molecules. Whether it be characterization of a candidate drug in serum binding assays or detailed study of antigen-antibody interactions, equilibrium dialysis proves to be the most accurate method available. Equilibrium dialysis is inexpensive and easy to perform, the only instrumentation required is that used to quantify the compound of interest. Since the results of the assay are obtained under equilibrium conditions, the true nature of the interaction can be studied. Equilibrium dialysis also offers the ability to study low affinity interactions that are undetectable using other methods.

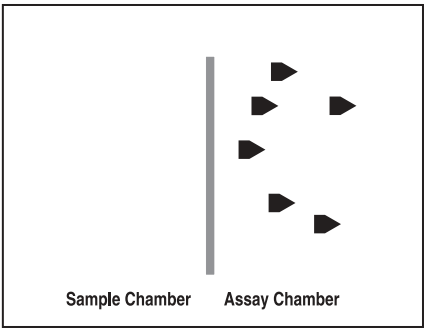
This guide offers an introduction to the technique of equilibrium dialysis and some examples of how this technique can be used in real world applications. There is also an introduction to the types of data analysis methods used to extract results from these types of experiments. Details of the wide range of equilibrium dialysis products offered by Harvard Bioscience can be found towards the back of this booklet.

Protocol

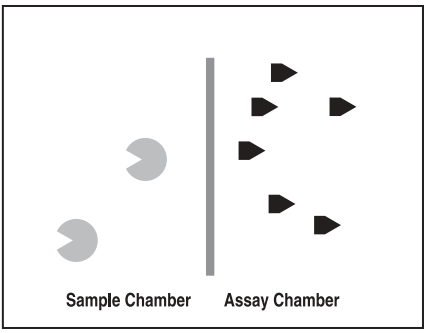
In a standard equilibrium dialysis assay you begin with two chambers separated by a dialysis membrane. The molecular weight cut off (MWCO) of this membrane is chosen such that it will retain the receptor component of the sample (the element which will bind the ligand).



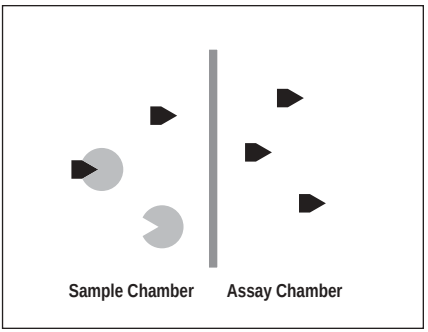
A known concentration and volume of ligand is placed into one of the chambers. The ligand is small enough to pass freely through the membrane.



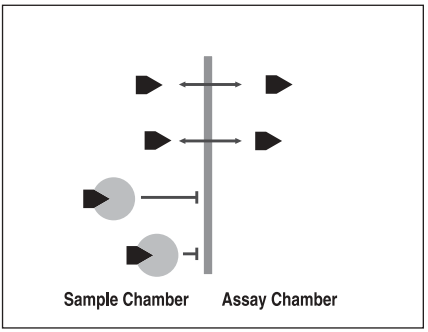
A known concentration of receptor is then placed in the remaining chamber in an equivalent volume to that placed in the first chamber.



As the ligand diffuses across the membrane some of it will bind to the receptor and some will remain free in solution. The higher the affinity of the interaction, the higher the concentration of ligand that will be bound at any time.



Diffusion of the ligand across the membrane and binding of the ligand continues until equilibrium has been reached. At equilibrium, the concentration of ligand free in solution is the same in both chambers. In the receptor chamber, however, the overall concentration is higher due to the bound-ligand component.



The concentration of free ligand in the ligand chamber can then be used to determine the binding characteristics of the samples as described in the next section.

Analysis

Equilibrium Dialysis can be used in a wide variety of experiments and the methods used to analyze the resulting data can vary just as widely. This section serves as an introduction to the types of data analysis tools used to interpret experimental data generated using equilibrium dialysis.

The type of assay typically performed using equilibrium dialysis falls under the category of saturation binding experiments. In this case the equilibrium binding of various concentrations of the receptor and ligand is measured. The relationship between binding and ligand concentration is then used to determine the number of binding sites, Bmax, and the ligand affinity, kd. Because this kind of experimental data used to be analyzed with Scatchard plots, they are sometimes called “Scatchard experiments”.

Analysis of Ligand Binding Data

Analysis of ligand binding experiments is based on a simple model, called the law of mass action. This model assumes that binding is reversible.

$$Receptor + Ligand \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} Receptor \bullet Ligand$$

Binding occurs when ligand and receptor collide due to diffusion, and when the collision has the correct orientation and enough energy. The rate of association is:

$$[Ligand] \cdot [Receptor] \cdot k_{on}$$

[ ] denotes concentration

The association rate constant (kon) is expressed in units of M-1min-1 . Once binding has occurred, the ligand and receptor remain bound together for a random amount of time. The probability of dissociation is the same at every instant of time. The receptor doesn't "know" how long it has been bound to the ligand. The rate of dissociation is:

$$[Ligand \bullet Receptor] \cdot k_{off}$$

The dissociation constant koff is expressed in units of min-1. After dissociation, the ligand and receptor are the same as at they were before binding. If either the ligand or receptor is chemically modified, then the binding does not follow the law of mass action. Equilibrium is reached when the rate at which new ligand-receptor complexes are formed equals the rate at which the ligand-receptor complexes dissociate. At equilibrium:

$$[Ligand] \cdot [Receptor] \cdot k_{on} = [Ligand \bullet Receptor] \cdot k_{off}$$

Rearrange that equation to define the equilibrium dissociation constant kd. Define the equilibrium dissociation constant, kd to equal koff/kon, which is in molar units. In enzyme kinetics, this is called the Michaelis-Menten constant, KM.

$$\frac{[Ligand]\cdot[Receptor]}{[Ligand \bullet Receptor]} = \frac{k_{off}}{k_{on}} = k_d$$

The kd has a meaning that is easy to understand. Set [Ligand] equal to kd in the equation above. The kd terms cancel out, and you'll see that [Receptor]/[Ligand • Receptor]=1, so [Receptor] equals [Ligand • Receptor]. Since all the receptors are either free or bound to ligand, this means that half the receptors are free and half are bound to ligand. In other words, when the concentration of ligand equals the kd, half the receptors will be occupied at equilibrium. If the receptors have a high affinity for the ligand, the kd will be low, as it will take a low concentration of ligand to bind half the receptors.

Don't mix up kd, the equilibrium dissociation constant, with koff, the dissociation rate constant. They are not the same, and aren't even expressed in the same units.

Variable	Name	Units
k_{on}	Association rate constant or on-rate constant	$M^{-1}min^{-1}$
k_{off}	Dissociation rate constant or off-rate constant	min^{-1}
k_d	Equilibrium dissociation constant	M

Fractional occupancy is the fraction of all receptors that are bound to ligand.

$$Fractional\ Occupancy = \frac{[Ligand \bullet Receptor]}{[Total\ Receptor]} = \frac{[Ligand \bullet Receptor]}{[Receptor] + [Ligand \bullet Receptor]}$$

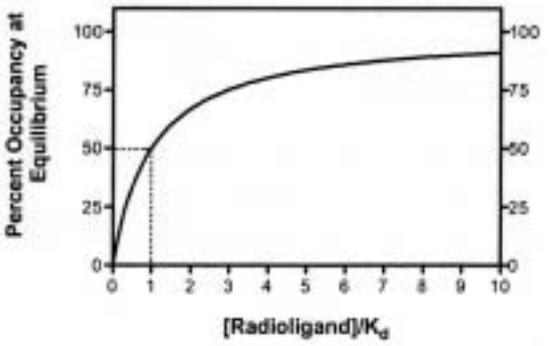
This equation can be more clearly represented as:

$$Fractional\ Occupancy = \frac{[Ligand]}{[Ligand] + K_d}$$

This equation assumes equilibrium. To make sense of it, think about a few different values for [Ligand].

[Ligand]	Fractional Occupancy
0	0
$1 \bullet k_d$	50%
$4 \bullet k_d$	80%
$9 \bullet k_d$	90%
$99 \bullet k_d$	99%

This becomes even clearer in graphical form.



Note that when [Ligand]=kd, fractional occupancy is 50%.

Although termed a “law”, the law of mass action is simply a model that can be used to explain some experimental data. Because it is so simple, the model is not useful in all situations. The model assumes:

- All receptors are equally accessible to ligands.
- Receptors are either free or bound to ligand. It doesn’t allow for more than one affinity state, or states of partial binding.
- Binding does not alter the ligand or receptor.
- Binding is reversible.

Despite its simplicity, the law of mass action has proven to be very useful in describing many aspects of receptor pharmacology and physiology.

Linear Regression

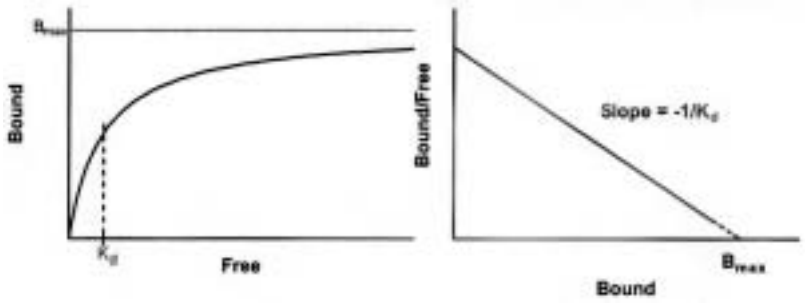
Linear Regression: Introduction

In the days before nonlinear regression programs (eg. Graphpad Prism) were widely available,scientists transformed data into a linear form, and then analyzed the data by linear regression.

Linear regression analyzes the relationship between two variables, X and Y. For each subject (or experimental unit), you know both X and Y and you want to find the best straight line through the data. In some situations, the slope and/or intercept have a scientific meaning. In other cases, you use the linear regression line as a standard curve to find new values of X from Y, or Y from X. In general, the goal of linear regression is to find the line that best predicts Y from X. Linear regression does this by finding the line that minimizes the sum of the squares of the vertical distances of the points from the line.

Linear Regression: The Scatchard Plot

There are several ways to linearize binding data, including the methods of Lineweaver-Burke and Eadie-Hofstee. However, the most popular method to linearize binding data is to create a Scatchard plot, as shown in the right panel below.

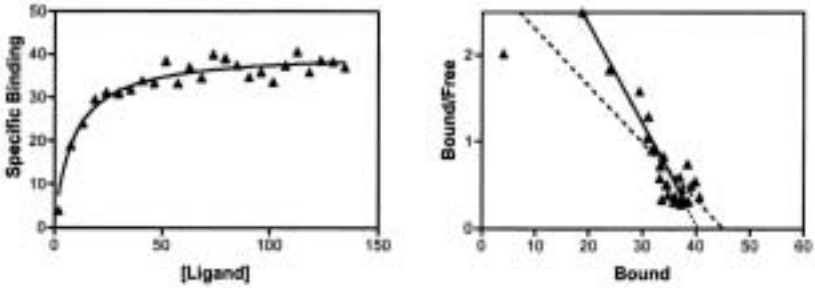


In this plot, the X-axis is specific binding and the Y-axis is specific binding divided by free ligand concentration. It is possible to estimate the Bmax and kd from a Scatchard plot (Bmax is the X intercept; kd is the negative reciprocal of the slope). However, the Scatchard transformation distorts the experimental error, and thus violates several assumptions of linear regression. The Bmax and kd values you determine by linear regression of Scatchard transformed data may be far from their true values.

Linear Regression: *Analysis*

The problem with this method is that the transformation distorts the experimental error. Linear regression assumes that the scatter of points around the line follows a Gaussian distribution and that the standard deviation is the same at every value of X. These assumptions are rarely true after transforming data. Furthermore, some transformations alter the relationship between X and Y. For example, in a Scatchard plot the value of X (bound) is used to calculate Y (bound/free), and this violates the assumption of linear regression that all uncertainty is in Y while X is known precisely. It doesn't make sense to minimize the sum of squares of the vertical distances of points from the line, if the same experimental error appears in both X and Y directions. Since the assumptions of linear regression are violated, the values derived from the slope and intercept of the regression line are not the most accurate determinations of the variables in the model. Considering all the time and effort you put into collecting data, you want to use the best possible technique for analyzing your data. Nonlinear regression produces the most accurate results.

The graph below shows the problem of transforming data. The left panel shows data that follows a rectangular hyperbola (binding isotherm). The right panel is a Scatchard plot of the same data. The solid curve on the left was determined by nonlinear regression. The solid line on the right shows how that same curve would look after a Scatchard transformation. The dotted line shows the linear regression fit of the transformed data. Scatchard plots can be used to determine the receptor number (Bmax, determined as the X-intercept of the linear regression line) and dissociation constant (kd, determined as the negative reciprocal of the slope). Since the Scatchard transformation amplified and distorted the scatter, the linear regression fit does not yield the most accurate values for B_{max} and k_d.



Don't use linear regression just to avoid using nonlinear regression. Fitting curves with nonlinear regression is not difficult using software programs such as Graphpad Prism. Although it is usually inappropriate to analyze transformed data, it is often helpful to display data after a linear transform. Many people find it easier to visually interpret transformed data. Even if you analyze your data with nonlinear regression, it may make sense to display the results of a linear transform.

Non-Linear Regression

Non-Linear Regression: *Introduction*

Linear regression is described in every statistics book, and is performed by every statistics program. Nonlinear regression is mentioned in only a few books, and is not performed by all statistics programs. From a mathematician's point of view, the two procedures are vastly different. From a scientist's point of view, however, the two procedures are very similar. In many fields of science, nonlinear regression is used far more often than linear regression. A line is described by a simple equation that calculates Y from X, slope and intercept. The purpose of linear regression is to find values for the slope and intercept that define the line that comes closest to the data. More precisely, it finds the line that minimizes the sum of the square of the vertical distances of the points from the line. The equations used to do this can be derived with no more than high-school algebra (shown in many statistics books). Put the data in, and the answers come out. There is no chance for ambiguity. You could even do the calculations by hand, if you wanted to.

Nonlinear regression is more general than linear regression. It fits data to any equation that defines Y as a function of X and one or more parameters. It finds the values of those parameters that generate the curve that comes closest to the data. More precisely, nonlinear regression finds the values of the parameters that generates a curve that minimizes the sum of the squares of the vertical distances of the data points from the curve.

Except for a few special cases, it is not possible to directly derive an equation to compute the best-fit values from the data. Instead nonlinear regression requires a computationally intensive, iterative approach. You can't really follow the mathematics of nonlinear regression unless you are familiar with matrix algebra. But these complexities only pertain to performing the calculations, which can be performed easily with non-linear regression software (like GraphPad Prism). Using nonlinear regression to analyze data is only slightly more difficult than using linear regression. Your choice of linear or nonlinear regression should be based on the model you are fitting. Don't use linear regression just to avoid using nonlinear regression.

Non-Linear Regression: *Sum-of-Squares*

The goal of nonlinear regression is to adjust the values of the variables in the model to find the curve that best predicts Y from X. More precisely, the goal of regression is to minimize the sum of the squares of the vertical distances of the points from the curve. Why minimize the sum of the squares of the distances? Why not simply minimize the sum of the actual distances?

If the random scatter follows a Gaussian distribution, it is far more likely to have two medium size deviations (say 5 units each) than to have one small deviation (1 unit) and one large (9 units). A procedure that minimized the sum of the absolute value of the distances would have no preference over a curve that was 5 units away from two points and one that was 1 unit away from one point and 9 units from another. The sum of the distances (more precisely, the sum of the absolute value of the distances) is 10 units in each case. A procedure that minimizes the sum of the squares of the distances prefers to be 5 units away from two points (sum-of-squares = 25) rather than 1 unit away from one point and 9 units away from another (sum-of-squares = 82). If the scatter is Gaussian (or nearly so), the curve determined by minimizing the sum-of-squares is most likely to be correct.

Non-Linear Regression: *Analysis*

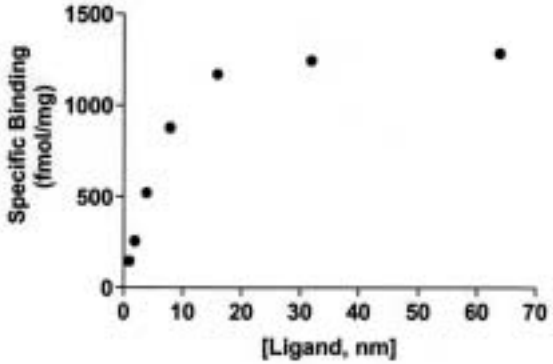
While the mathematical details of non-linear regression are quite complicated, the basic idea is pretty easy to understand. Every nonlinear regression method follows these steps:

1. Start with an initial estimated value for each variable in the equation.
2. Generate the curve defined by the initial values. Calculate the sum-of-squares (the sum of the squares of the vertical distances of the points from the curve).
3. Adjust the variables to make the curve come closer to the data points. There are several algorithms for adjusting the variables, as explained below.
4. Adjust the variables again so that the curve comes even closer to the points. Repeat.

5. Stop the calculations when the adjustments make virtually no difference in the sum-of-squares.
6. Report the best-fit results. The precise values you obtain will depend in part on the initial values chosen in step 1 and the stopping criteria of step 5. This means that repeat analyses of the same data will not always give exactly the same results.

Step 3 is the only difficult one. Prism (and most other nonlinear regression programs) uses the method of Marquardt and Levenberg, which blends two other methods, the method of linear descent and the method of Gauss-Newton.

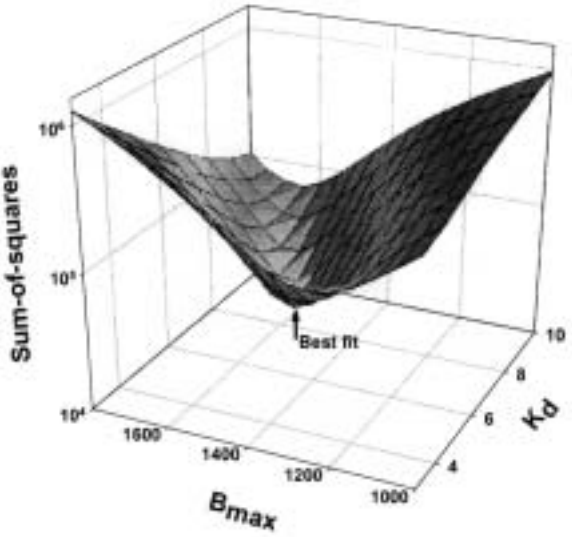
The best way to understand these methods is to follow an example. Here are some data to be fit to a typical binding curve (rectangular hyperbola).



You want to fit a binding curve to determine B_{max} and kd using the equation:

$$Y = \frac{B_{max} \cdot X}{k_d + X}$$

How can you find the values of Bmax and kd that fit the data best? You can generate an infinite number of curves by varying Bmax and kd. For each of the generated curves, you can compute the sum-of-squares to assess how well that curve fits the data. The following graph illustrates the situation.



The X- and Y-axes correspond to two variables to be fit by nonlinear regression (Bmax and kd in this example). The Z-axis is the sum-of-squares. Each point on the surface corresponds to one possible curve. The goal of nonlinear regression is to find the values of Bmax and kd that make the sum-of-squares as small as possible (to find the bottom of the valley).

The method of linear descent follows a very simple strategy. Starting from the initial values try increasing each parameter a small amount. If the sum-of-squares goes down, continue. If the sum-of-squares goes up, go back and decrease the value of the parameter instead. You've taken a step down the surface. Repeat many times. Each step will usually reduce the sum-of-squares. If the sum-of-squares goes up instead, the step must have been so large that you went past the bottom and back up the other side. If this happens, go back and take a smaller step. After repeating these steps many times, you'll reach the bottom.

The Gauss-Newton method is a bit harder to understand. As with the method of linear descent, start by computing how much the sum-of-squares changes when you make a small change in the value of each parameter.

This tells you the slope of the sum-of-squares surface at the point defined by the initial values. If the equation really were linear, this is enough information to determine the shape of the entire sum-of-squares surface, and thus calculate the best-fit values of Bmax and kd in one step. With a linear equation, knowing the slope at one point tells you everything you need to know about the surface, and you can find the minimum in one step. With nonlinear equations, the Gauss-Newton method won't find the best-fit values in one step, but that step usually improves the fit. After repeating many iterations, you reach the bottom.

This method of linear descent tends to work well for early iterations, but works slowly when it gets close to the best-fit values (and the surface is nearly flat). In contrast, the Gauss-Newton method tends to work badly in early iterations, but works very well in later iterations. The two methods are blended in the method of Marquardt (also called the Levenberg-Marquardt method). It uses the method of linear descent in early iterations and then gradually switches to the Gauss-Newton approach. Graphpad Prism, like most programs, uses the Marquardt method for performing nonlinear regression.

Example

The following example is one possible method of analysis for data from a ligand binding experiment.

In this experiment, 1ml samples of a 50,000 Da Protein (5.0 mg/ml) are allowed to come to equilibrium with 1ml volumes of a ligand solution of several concentrations. The concentrations of the ligand solutions used in the experiment are shown in the table below ($[Ligand]_{total}$).

$[Ligand]_{total}$ (mmol)	$[Ligand]_{free}$ (mmol)	$[Ligand]_{bound}$ (mmol)
0.01	0.005	0.005
0.02	0.011	0.009
0.05	0.030	0.020
0.08	0.046	0.029
0.10	0.062	0.038
0.15	0.104	0.046
0.20	0.143	0.057
0.40	0.332	0.068
0.70	0.623	0.077
1.00	0.922	0.078
1.25	1.170	0.080

Once equilibrium has been reached the concentration of free ligand is measured ([Ligand]free) and the concentration of bound ligand can be determined ([Ligand]bound). The experimental results for this example are presented in the table above.

At this stage in the experiment a decision must be made regarding how the experimental data will be analyzed. In this case we will plot a binding isotherm of the data, use non-linear regression to find the best-fit line for this data (and hence determine Bmax and Kd). For ease of visual interpretation we will then perform a Scatchard transformation on the resultant best-fit line data.

Generating a binding isotherm for this data involves plotting ligand concentration ($[Ligand]_{free}$) in millimoles on the X-axis against binding coefficient (B) on the Y-axis. The binding coefficient is given by:

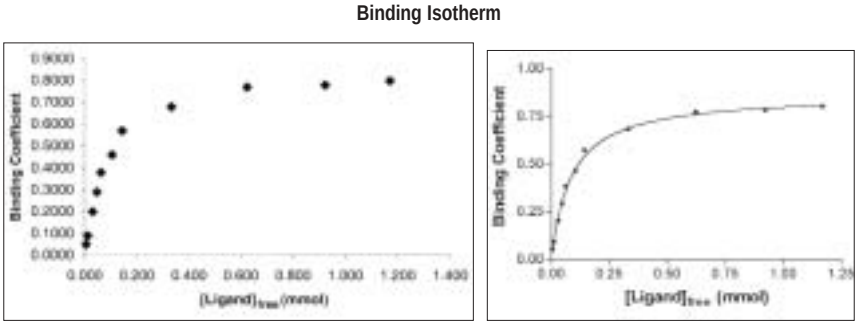
$$B = \frac{[Ligand]_{bound}}{[Protein]_{total}}$$

The concentration of protein is the same in each case, 0.1 mmol.

$[Ligand]_{free}$ (mmol)	Binding Coefficient
0.005	0.0500
0.011	0.0900
0.030	0.2000
0.046	0.2900
0.062	0.3800
0.104	0.4600
0.143	0.5700
0.332	0.6800
0.623	0.7700
0.922	0.7800
1.170	0.8000

We then use non-linear regression (Graphpad Prism) to find the best-fit line for the data.

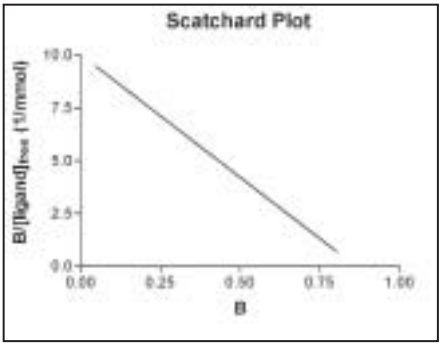
This can then be plotted:



When using a software package such as Prism, Bmax and Kd are determined automatically. When this facility is not available it is possible to determine these values from a Scatchard plot, although this will be less accurate (as discussed in the linear regression section). The data obtained from the non-linear regression can be put through a Scatchard transformation to generate a linear plot.

The equation of this line is given by:

$$y = -11.61x + 10.03$$



The Scatchard equation is:

$$B/L = n/Kd - B/Kd$$

Where:

B = $[Ligand]bound/[Protein]_{total}$

L = $[Ligand]_{free}$

n = number of ligands/macromolecule, i.e.the stoichiometry

Kd = the dissociation constant

Thus K_d can be determined as the negative reciprocal of the slope of the line and B_{max} is given by the X-intercept.

In this case Kd is 0.086 mmol (8.6 x 10-5M) and Bmax is 0.864.

Additional Reading

To learn more about how nonlinear regression works, we recommend reading:

96 Well Equilibrium Dialyzer™
Kariv I., Cao H., Oldengurg K, (May 2001) Development of a High Throughput Equilibrium Dialysis Method. Journal of Pharmaceutical Sciences Vol. 90, No,5, 580-587.

Dispo Equilibrium Dialyzer™
Three-dimensional Structure of Guanylyl Cyclase Activating Protein-2, a Calcium-sensitive Modulator of Photoreceptor Guanylyl Cyclases James B. Ames, Alexander M. Dizhoor, Mitsuhiko Ikura, Krzysztof Palczewski, and Lubert Stryer the journal of biological chemistry Vol. 274, No. 27, Issue of July 2, pp. 19329-19337, 1999

Chapter 15 of Numerical Recipes in C, Second Edition, WH Press, et. Al., Cambridge Press, 1992.

Chapter 10 of Primer of Applied Regression and Analysis of Variance, SA Glantz and BK Slinker, McGraw-Hill, 1990.

Analyzing Data with GraphPad Prism, H.J. Motulsky, GraphPad Software, 1999. Available at www.graphpad.com.

Products

Dispo-Equilibrium Dialyzer™

Harvard/AmiKa's Dispo-Equilibrium Dialyzer is a single-use product for interaction studies. The Dispo-Equilibrium Dialyzer is leak-proof and provides high sample recovery (almost 100 percent). This system is designed for one-time use with samples such as radiolabeled compounds, avoiding the hassle associated with cleaning the dialyzer after use.
Each chamber has a capacity of up to 75µl. The Dispo-Equilibrium Dialyzer utilizes high-quality regenerated cellulose membranes with MWCO's of 5,000 or 10,000 Daltons. Sample recovery is very easy through centrifugation or via removal with micropipettes.

APPLICATIONS

- **Protein binding assays**
- **Protein-drug binding assays**
- **Receptor binding assays**
- **Ligand binding assays**
- **Protein-protein interations**
- **Protein-DNA interactions**

ADVANTAGES

- **Easy to use**
- **Disposable - no clean up**
- **Small sample volumes: 25 to 75µl each chamber**
- **Rapid dialysis due to ultra-thin membrane**
- **Membrane MWCOs of 5K and 10K Daltons**
- **High-quality regenerated cellulose membranes**
- **Leak-proof**

Dispo-Equilibrium Dialyzer			
Membrane MWCO (Daltons)	**Qty. of 25**	**Qty. of 50**	**Qty. of 100**
5,000	**MB 74-2204**	**MB 74-2200**	**MB 74-2201**
10,000	**MB 74-2205**	**MB 74-2202**	**MB 74-2203**

Catalog No.	Description	Quantity
MB 74-2222	Pipette Tips for Loading/Unloading	100

Products

Micro-Equilibrium Dialyzer™

The Micro-Equilibrium Dialyzer is a unique equilibrium dialysis chamber for small samples (25 to 500µl). Due to the small volume of the chamber, very small amounts of sample are required for protein binding assays. Two chambers of equivalent volume are joined together with a membrane between them, as shown. When dialysis is complete the chambers can be opened at each end to extract the sample for analysis. The entire system can also be placed in a thermostat for temperature-controlled dialysis.
The Micro-Equilibrium Dialyzer can also be used with three chambers instead of two. One of the main advantages of using this configuration is that the results can be obtained without waiting for equilibrium to be reached, thus reducing the assay time. This is achieved by placing the assay compound in the central chamber; the binding component in one of the terminal chambers and control buffer, containing neither component, in the remaining chamber. Comparing the concentration of the assay compound in the two terminal chambers will then yield information on the binding
The receptor element is placed in one chamber (the sample chamber) while the other chamber (the assay chamber) contains an equivalent volume of ligand solution. When equilibrium has been reached the concentration of the ligand in the assay chamber can be measured and analyzed to obtain the results of the assay.
When th ligand is free in solution it can readily pass through the membrane, but when it is complexed it is too large and is retained by the membrane.

APPLICATIONS

- **Protein binding assays**
- **Protein-drug binding assays**
- **Receptor binding assays**
- **Ligand binding assays**
- **Protein-protein interations**
- **Protein-DNA interactions**

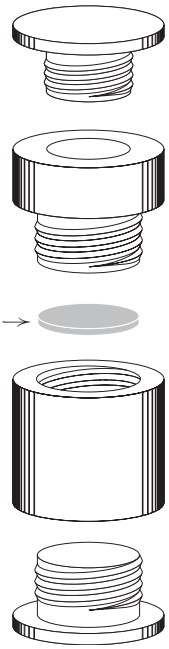
ADVANTAGES

- **Easy to use**
- **Leak-proof**
- **Reusable**
- **Available for a range of sample sizes**
- **Membranes available with MWCO's to suit almost any application**
- **Autoclaveable**
- **Low protein binding**
- **High sample recovery**
- **Made of Teflon – totally inert**

Products

Micro-Equilibrium Dialyzer™ (Continued)

2-Chamber System



Micro-Equilibrium Dialyzers			
Volume per Chamber (µl)	**Total Volume (µl)**	**Qty. of 1**	**Qty. of 5**
25	50	**MB 74-1606**	**MB 74-1600**
50	100	**MB 74-1607**	**MB 74-1601**
100	200	**MB 74-1608**	**MB 74-1602**
250	500	**MB 74-1609**	**MB 74-1603**
500	1,000	**MB 74-1610**	**MB 74-1604**
Additional Chambers for 3-Chamber System			
25	–	**MB 74-1619**	**MB 74-1620**
50	–	**MB 74-1611**	**MB 74-1615**
100	–	**MB 74-1612**	**MB 74-1616**
250	–	**MB 74-1613**	**MB 74-1617**
500	–	**MB 74-1614**	**MB 74-1618**

Ultra-Thin Membranes for Micro-Equilibrium Dialyzer		
Membrane MWCO (Daltons)	**Qty. of 24**	**Qty. of 96**
For Use with 25, 50 and 100µl Volume Chambers		
5,000	MB 74-1704	MB 74-1700
10,000	MB 74-1705	MB 74-1701
For Use with 250 and 500µl Volume Chambers		
5,000	MB 74-1706	MB 74-1702
10,000	MB 74-1707	MB 74-1703

Other membranes available:

- Cellulose acetate MWCO 100K Daltons to 300K Daltons
- Regenerated Cellulose MWCO 1K Daltons to 50K Daltons
- Polycarbonate .01µm to .6 µm Pore Size

Products

Equilibrium Dialyzer-96™

The Equilibrium Dialyzer-96 is a novel product for the simultaneous assay of 96 samples. Each well in this system has a separate membrane and thus eliminates the possibility of sample cross-contamination. Reproducibility is very high across the different wells of the Equilibrium Dialyzer-96 and sample recovery is excellent. Wells are sealed with 8-cap strips. Thus a row of wells, or all 96 wells can be used depending on the specifications of the experiment. The Equilibrium Dialyzer-96 utilizes high-quality regenerated cellulose membranes available with MWCO's of 5,000 or 10,000 Daltons.

APPLICATIONS

- **Protein binding assays**
- **Protein-drug binding assays**
- **Receptor binding assays**
- **Ligand binding assays**
- **Protein-protein interations**
- **Protein-DNA interactions**

ADVANTAGES

- **96-well format**
- **Individual membrane for each well**
- **Small sample volumes: 50 to 200µl**
- **Ultra-thin regenerated cellulose membreanes**
- **Membranes are free of sulfur and other heavy metals**
- **High well-to-well reproducibility**
- **Excellent sample recovery (>95%)**

Catalog No.	Description	Quantity
MB 74-2330	Equilibrium Dialyzer-96 Plate, Membrane MWCO 5K Daltons	1
MB 74-2331	Equilibrium Dialyzer-96 Plate, Membrane MWCO 10K Daltons	1

Products

Plate Rotator

A Plate Rotator with variable rotation rates is available for use with Harvard/AmiKa's Equilibrium Dialyzer-96™. The Rotator speeds up the equilibrium dialysis process by keeping the sample in constant motion ensuring higher reproducibility of results.

Catalog No.	Description	Quantity
MB 74-2302	Plate Rotator, Single Plate	1
MB 74-2308	Plate Rotator, 8 Plates, Hybridization Oven	1

Products

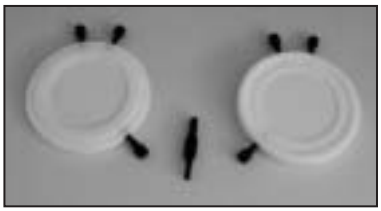
Multi-Equilibrium Dialyzer™

The Harvard/AmiKa Multi-Equilibrium Dialyzer provides highly standardized equilibrium dialysis conditions for up to 20 parallel assays. The instrument offers outstanding uniformity of:

- Membrane Area
- Sample Volume
- Degree of Agitation

The advantages of this system are that up to 20 cells can be used simultaneously for rapid dialysis under standardized conditions. Experiments conducted using the Multi-Equilibrium Dialyzer are extremely reproducible and leak-proof and can be performed at a constant temperature.

The dialyzer cells are made of Teflon, an extremely inert material, and will not interfere with the samples. Multiple cell systems are available (5, 10, 15, 20 cells) at various cell volumes (0.25, 1.0, 2.0 & 5.0ml). The unit can be sterilized by auto-claving and the cells can be filled easily with a filling clamp.



Products

APPLICATIONS

- **Protein binding assays**
- **Protein-drug binding assays**
- **Receptor binding assays**
- **Ligand binding assays**
- **Protein-protein interations**
- **Protein-DNA interactions**

ADVANTAGES

- **Easy to use**
- **Leak-proof**
- **Reproducible**
- **Fast dialysis times**
- **Available for a range of sample sizes**
- **Autoclavable**
- **Low protein binding**
- **High sample recovery**
- **Made of Teflon – totally inert**

Catalog No.	Description	Quantity
Multi-Equilibrium Dialyzer Systems		
MB 74-1800	Complete Multi-Equilibrium Dialyzer System	
	- Ready-to-Use Teflon Macro Dialysis Cells (1ml) with Large Surface Area	20
	- Variable Speed Drive Unit for 20 Cells	1
	- Stand	1
	- Carriers for 5 Teflon Dialysis Cells	4
	- Dialysis Membranes MWCO 10K Daltons with Very High Permeability	200
Membranes for Multi-Equilibrium Dialyzer		
MB 74-2100	MWCO 5K Daltons	200
MB 74-2101	MWCO 10K Daltons	200
MB 74-2102	MWCO 10K Daltons with Very High Permeability	200
Multi-Equilibrium Dialyzer Individual Components		
MB 74-1913	Filling Clamp	1
MB 74-1901	Emptying Stoppers	5
MB 74-1914	Black Stoppers	32
MB 74-1907	Micro Teflon Dialysis Cells (0.2ml)	5
MB 74-1903	Macro Teflon Dialysis Cells (1ml)	5
MB 74-1904	Macro Teflon Dialysis Cells (2ml)	5
MB 74-1905	Macro Teflon Dialysis Cells (5ml)	5
MB 74-1906	Macro Teflon Dialysis Cells with	5Large Surface Area (1ml)

Products

Graphpad Prism

GraphPad Prism combines nonlinear regression (curve fitting), basic biostatistics, and scientific graphing. Prism's unique design will help you efficiently analyze, graph, and organize your experimental data. Prism helps you in many ways:

Fit curves with nonlinear regression. For many labs, nonlinear regression is the most commonly used data analysis technique. No other program streamlines (and teaches) curve fitting like Prism.

Perform statistics. Prism makes it easy to perform basic statistical tests commonly used by laboratory researchers and clinicians. Prism does not take the place of heavy duty statistics programs. Prism offers a complete set of statistical analyses up to two-way ANOVA, including analysis of contingency tables and survival curves. Prism does not perform ANOVA higher than two-way, or multiple, logistic or proportional hazards regression.

Create scientific graphs. Prism makes a wide variety of 2D scientific graphics. Included are all the features that scientists need including automatic calculation of error bars, Greek letters, log axes, discontinuous axes and much more.

Organize your work. Prism's unique organization helps you stay organized and lets you carefully track how all your data are analyzed. Your data and files are linked into one organized folder so it is always easy to retrace your steps.

Catalog No.	Description	Quantity
MB 74-2310	Graphpad Prism® (Windows)	1
MB 74-2311	Graphpad Prism® (Mac)	1


HARVARD
APPARATUS
9511-050