
Version 6.2

FitAll

nonlinear regression analysis

Binding Curves Function Guide



Copyright © 1984 .. 2009 by MTR Software
All rights reserved.

Published by

MTR Software

77 Carlton Street, Suite 808
Toronto ON Canada
M5B 2J7

www.fitall.com

support@fitall.com

416-596-1499

FitAll, the FitAll Function Libraries, the FitAll Getting Started Guide, the FitAll Reference Guide, the FitAll Programmer's Guide and the FitAll Function Guides are copyrighted and sold with the understanding that they will be used either on a single computer or by one individual, whichever is most appropriate for the purchaser.

When you purchase FitAll, you purchase a SINGLE USER LICENCE.

Selling (or giving) copies of FitAll, its Function Libraries or Guides to others is an infringement of the copyright. That means it is illegal!.

"FitAll" and "fitting solutions" are trade marks of MTR Software.

Contents

Introduction	1
Function Reference	3
Ftn 0201: Langmuir Adsorption Isotherm.....	4
Ftn 0202: Coupled Saturation Curves (Zero Origin).....	6
Ftn 0203: Coupled Saturation Curves (Non Zero Origin).....	9
Ftn 0204: Cooperative Saturation Curve.....	12
Ftn 0205: Uncoupled Saturation Curves (Zero Origin).....	14
Ftn 0206: Uncoupled Saturation Curves (Non zero Origin).....	16
Ftn 0207: DNA-DRUG Binding: Multi-Site, Single Experiment; f's as parameters	18
Ftn 0208: DNA-DRUG Binding: Multi-Site, Multi-Experiment; f's as variables	20
Ftn 0209: DNA-DRUG Binding: Multi-Site, Multi-Experiment; f's as constants	24
Appendix	29
Getting Help.....	30
Adding Functions to FitAll.....	31
Example 1: Using Functions 0207/8/9.....	32
Background	33
General Approach	34
Fitting Single Experiment Data	38
Fitting All the Data	52
Index	61

Introduction

This *FitAll*[™] **Binding Curves Function Guide** describes the functions contained in the **Binding Curves Functions Library** and has an appendix that explains how to get help from **MTR Software**.

[Function Reference](#) 

[Appendix](#) 

Function Reference

Overview

This section describes each of the functions in **FitAll's Binding Curves Functions Library**.

In most cases, a graph of the function is shown. These graphs were created using "typical" parameter and constant values.

The actual appearance of a function depends on the parameter and constant values and may look quite different from the illustrations shown.

Equation

Gives the equation and its variations. The variations are listed in order of increasing complexity.

Constants

Lists the constants, K, that are used in the function. The default values for the constants also are given.

Parameters

Lists the parameters, P, that are used in the function.

Multi-Fits

Describes the Multi-Fit functionality of "Multi-Fit enabled" functions.

Sample Applications

Gives examples of some situations in which the function is known to be used.

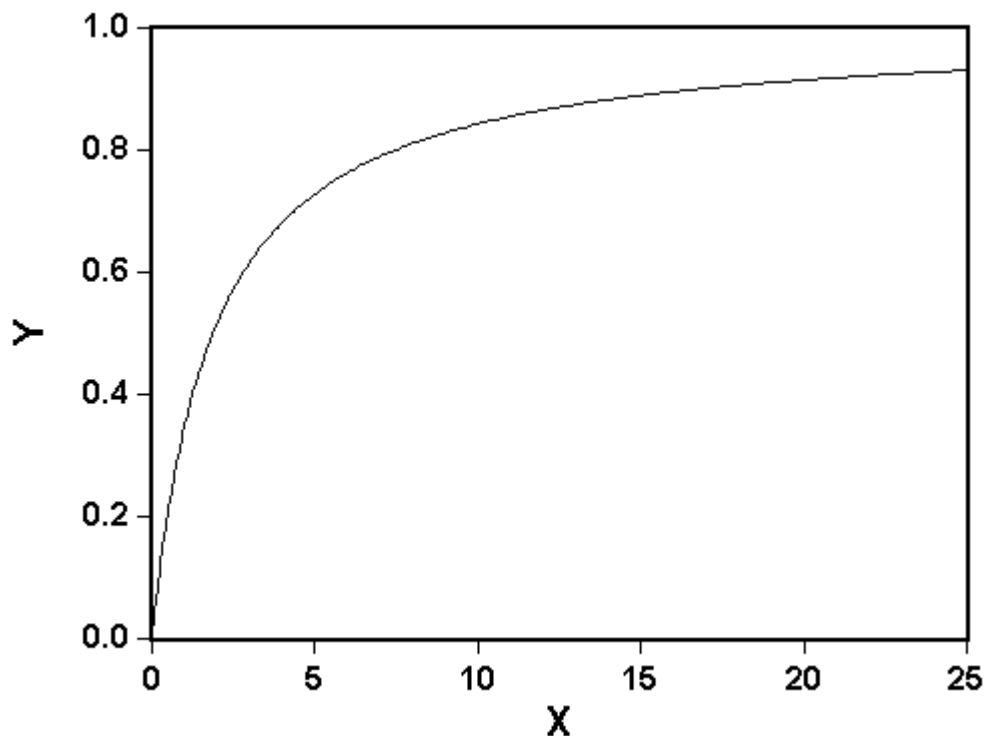
Remarks

Provides general comments and hints, and lists any known limitations or restrictions that should be observed when using the function.

Also see

Provides links or references to other related functions.

Ftn 0201: Langmuir Adsorption Isotherm



Equation

The function is:

$$Y = \frac{P1 * X}{(1 + P1 * X)}$$

in which

- Y is the measured response.
- X is the independent variable.

Parameters

Parameter	Name	Comments
P1	K langmuir	Langmuir Adsorption Constant

Sample Applications

- Quantify the degree of adsorption of a corrosion inhibitor onto a surface.

Remarks

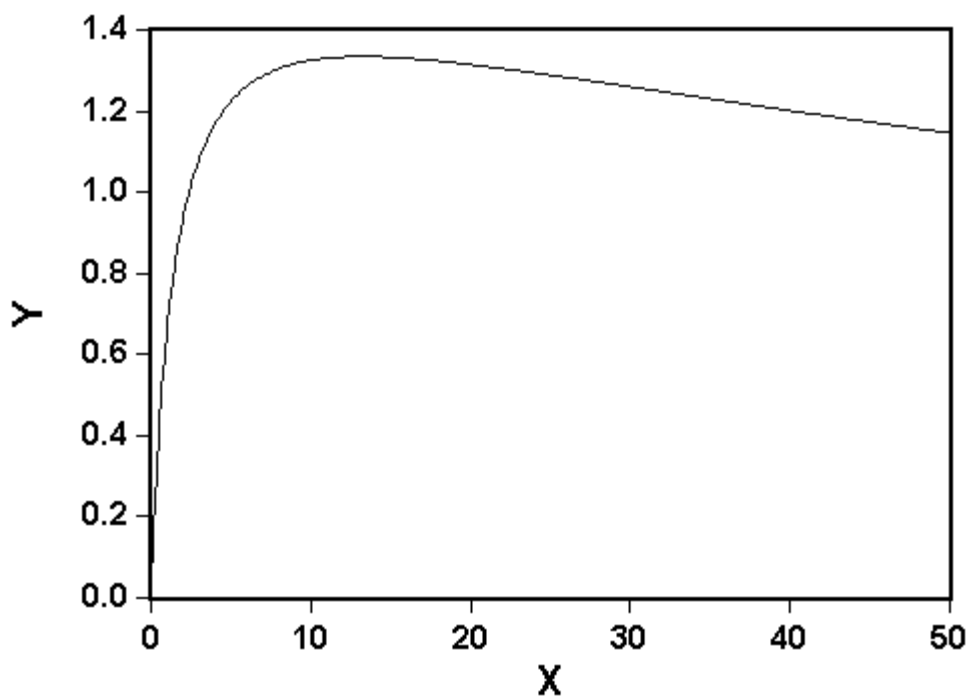
At fifty percent (50%) surface (site) coverage $X = 1/P1$.

At high values of X , the limiting value of Y is 1.0.

At $X = 0$, $Y = 0$.

When automatic initial estimates are made, **FitAll** assumes that the data are sorted on column number 1; that is, the X -values.

Ftn 0202: Coupled Saturation Curves (Zero Origin)



Equation

The general form of the function is:

$$Y = \frac{\sum_{i=1}^n \left(X^i * P_{2i} * \prod_{j=1}^i P_{2j-1} \right)}{1 + \sum_{i=1}^n \left(X^i * \prod_{j=1}^i P_{2j-1} \right)}$$

in which

- n is the number of coupled saturation curves. "n" can have values from 1 to 5.
- Y is the measured response.
- X is the independent variable, often the concentration of a substance.

Five variations of the function are available. For example:

- $$Y = \frac{P1 * P2 * X}{(1 + P1 * X)}$$

$$Y = \frac{(P1 * P2 * X + P1 * P3 * P4 * X^2)}{(1 + P1 * X + P1 * P3 * X^2)}$$

$$Y = \frac{(P1 * P2 * X + P1 * P3 * P4 * X^2 + P1 * P3 * P5 * P6 * X^3)}{(1 + P1 * X + P1 * P3 * X^2 + P1 * P3 * P5 * X^3)}$$

Parameters

Parameter	Name	Comments
P1	Q1	Equilibrium quotient (formation constant) for the first component.
P2	DeltaY1	Amplitude of Y for the first component. Positive values of DeltaY indicate that Y increases as the value of X increases. Negative values of DeltaY indicate that Y decreases as the value of X increases.
P3	Q2	Equilibrium quotient (formation constant) for the second component.
P4	DeltaY2	Amplitude of Y for the second component. Positive values of DeltaY indicate that Y increases as the value of X increases. Negative values of DeltaY indicate that Y decreases as the value of X increases.
P5	Q3	Equilibrium quotient (formation constant) for the third component.
P6	DeltaY3	Amplitude of Y for the third component.
P7	Q4	Equilibrium quotient (formation constant) for the fourth component.
P8	DeltaY4	Amplitude of Y for the fourth component.
P9	Q5	Equilibrium quotient (formation constant) for the fifth component.
P10	DeltaY5	Amplitude of Y for the fifth component.

Sample Applications

- Determining equilibrium (stability or formation) constants, Q_i , for the binding of a ligand or substrate to a metal ion, enzyme, or surface.
- Resolving equilibrium constants, Q_i , and rate constants, ΔY_i , for a coupled reaction from the apparent rate constant's dependence on X.

Remarks

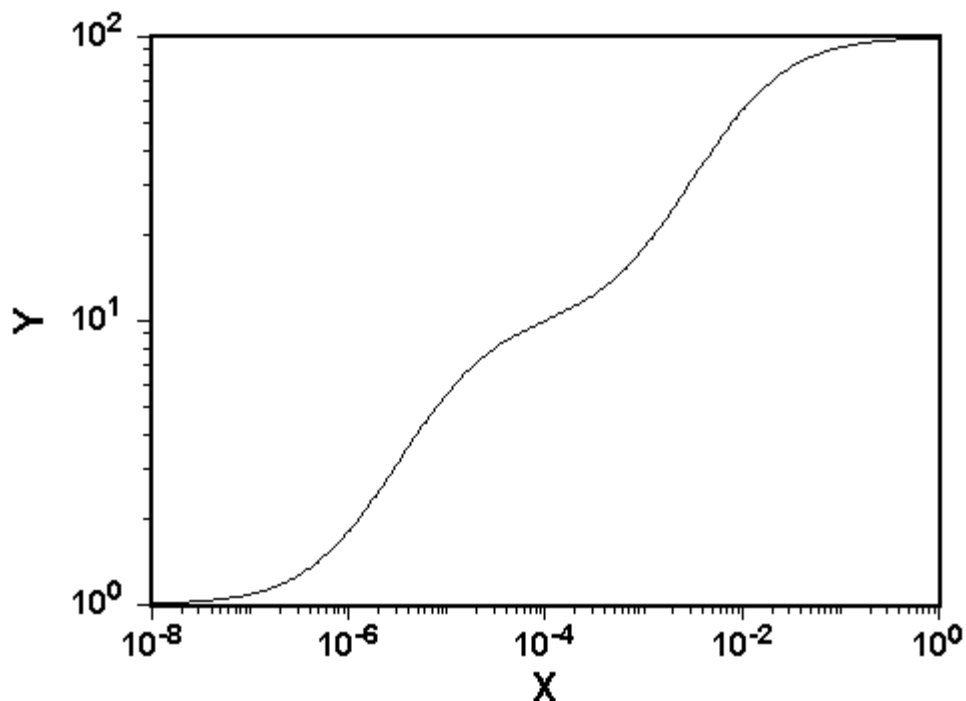
Similar to Ftn 0003 except that the limiting value of Y at high X is P2.

At X = 0, Y = 0.

When automatic initial estimates are made, **FitAll** assumes that the data are sorted on column number 1, that is, the X-values.

FitAll will calculate initial estimates only when you are fitting the data to one saturation curve.

Ftn 0203: Coupled Saturation Curves (Non Zero Origin)



Equation

The general form of the function is:

$$Y = \frac{P1 + \sum_{i=1}^n \left(X^i * P_{2i+1} * \prod_{j=1}^i P_{2j} \right)}{1 + \sum_{i=1}^n \left(X^i * \prod_{j=1}^i P_{2j} \right)}$$

in which

- Y is the measured response.
- X is the independent variable.
- n can have values from 1 to 5.

Five variations of the function are available. For example:

- $$Y = \frac{(P1 + P2 * P3 * X)}{(1 + P2 * X)}$$

$$Y = \frac{(P1 + P2 * P3 * X + P2 * P4 * P5 * X^2)}{(1 + P2 * X + P2 * P4 * X^2)}$$

$$Y = \frac{(P1 + P2 * P3 * X + P2 * P4 * P5 * X^2 + P2 * P4 * P6 * P7 * X^3)}{(1 + P2 * X + P2 * P4 * X^2 + P2 * P4 * P6 * X^3)}$$

Parameters

Parameter	Name	Comments
P1	Yo	Limiting value of Y when X is zero.
P2	Q1	Equilibrium quotient (formation constant) for the first component.
P3	DeltaY1	Amplitude of Y for the first component. Positive values of DeltaY indicate that Y increases as the value of X increases. Negative values of DeltaY indicate that Y decreases as the value of X increases.
P4	Q2	Equilibrium quotient (formation constant) for the second component.
P5	DeltaY2	Amplitude of Y for the second component. Positive values of DeltaY indicate that Y increases as the value of X increases. Negative values of DeltaY indicate that Y decreases as the value of X increases.
P6	Q3	Equilibrium quotient (formation constant) for the third component.
P7	DeltaY3	Amplitude of Y for the third component.
P8	Q4	Equilibrium quotient (formation constant) for the fourth component.
P9	DeltaY4	Amplitude of Y for the fourth component.
P10	Q5	Equilibrium quotient (formation constant) for the fifth component.
P11	DeltaY5	Amplitude of Y for the fifth component.

Sample Applications

- Determining equilibrium (stability or formation) constants, Q_i , for the binding of a ligand or substrate to a metal ion, enzyme, or surface.
- Resolving equilibrium constants, Q_i , and rate constants, ΔY_i , for a coupled reaction from the apparent rate constant's dependence on X.

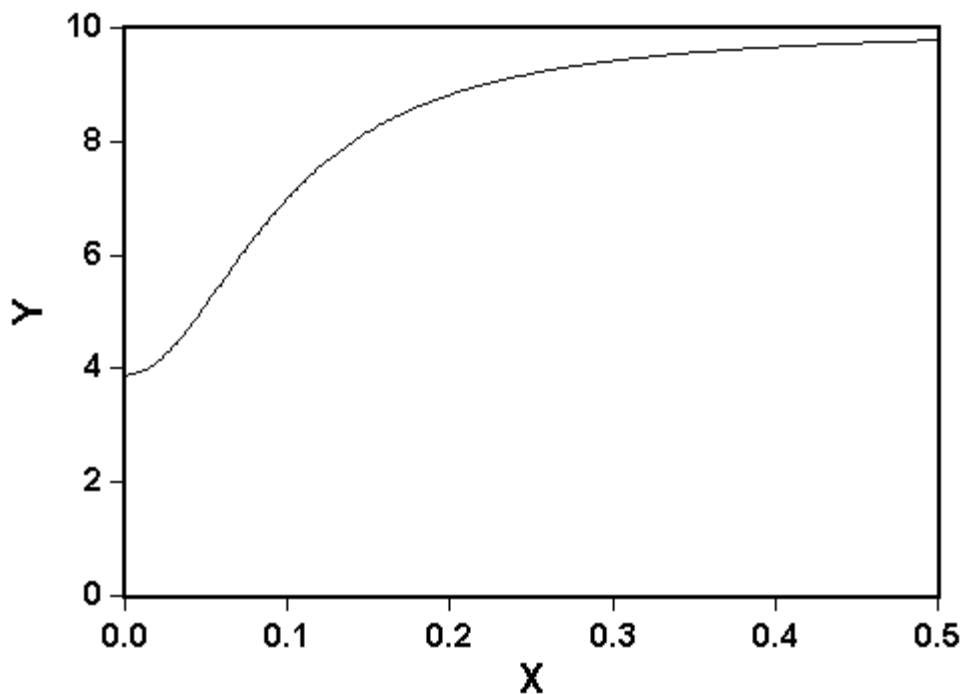
Remarks

Similar to [function 0201](#)^[4] and [function 0202](#)^[6], except that the limiting values of Y at high and low X are not constrained to any particular values.

When automatic initial estimates are made, **FitAll** assumes that the data are sorted on column number 1, that is., the X-values.

FitAll will calculate initial estimates only when you are fitting the data to one saturation curve.

Ftn 0204: Cooperative Saturation Curve



Equation

$$Y = \frac{(P3 + P2 * P1 * X^{P4})}{(1 + P1 * X^{P4})}$$

in which

- Y is the measured response.
- X is the independent variable, often the concentration of a substance.

Parameters

Parameter	Name	Comments
P1	K	Equilibrium quotient (formation constant).
P2	Yinf	Limiting value of Y when X is infinitely large.
P3	Yo	Limiting value of Y when X is zero.
P4	n	Exponent of X. In studies of substrate binding to an enzyme, n can sometimes be interpreted as the number of substrate molecules that are bound to the

Parameter	Name	Comments
		enzyme.

Sample Applications

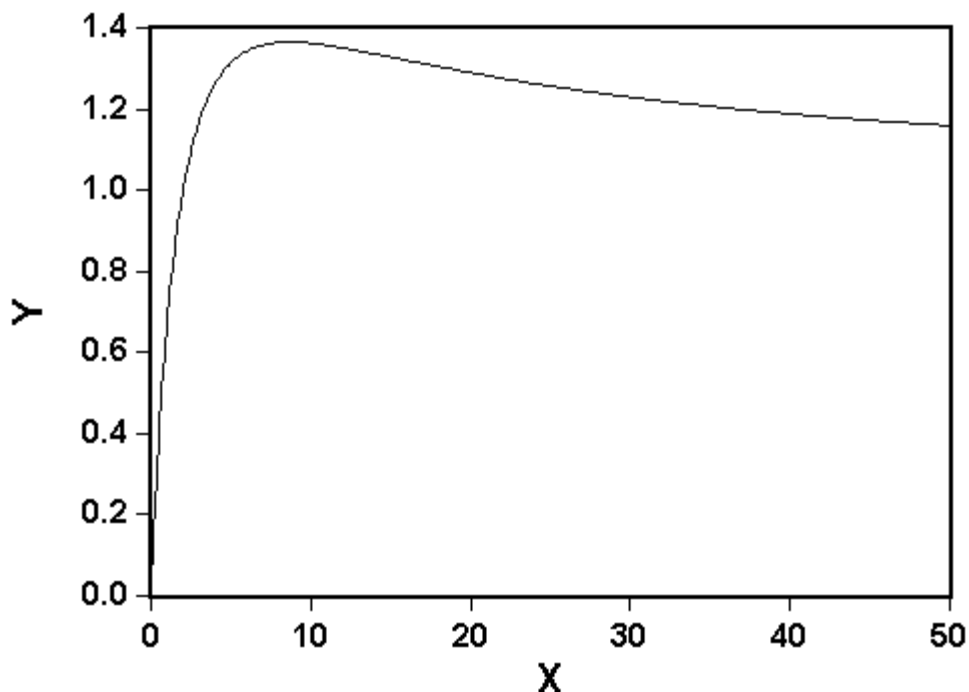
- Binding of substrates to enzymes.
- Adsorption of inhibitors on surfaces.

Remarks

The value of $P4, n$, is very sensitive to the quality of the data being fit, especially if it is larger than 2.0.

When automatic initial estimates are made, ***FitAll*** assumes that the data are sorted on column number 1; that is, the X-values.

Ftn 0205: Uncoupled Saturation Curves (Zero Origin)



Equation

The general form of the function is:

$$Y = \sum_{i=1}^n \left(\frac{P_{2i-1} * P_{2i} * X}{[1 + P_{2i-1} * X]} \right)$$

in which

- Y is the measured response.
- X is the independent variable, often the time in seconds.
- n is the number of uncoupled (parallel) saturation curves. "n" can have values from 1 to 10.

Ten variations of the function are available. For example:

- $$Y = \frac{P1 * P2 * X}{(1 + P1 * X)}$$

- $$Y = \frac{P1 * P2 * X}{(1 + P1 * X)} + \frac{P3 * P4 * X}{(1 + P3 * X)}$$

Parameters

Parameter	Name	Comments
P1	Q1	Equilibrium quotient (formation constant) for the first component
P2	DeltaY1	Amplitude of Y for the first component. Positive values of DeltaY indicate that Y increases as the value of X increases. Negative values of DeltaY indicate that Y decreases as the value of X increases.
P3	Q2	Equilibrium quotient (formation constant) for the second component
P4	DeltaY2	Amplitude of Y for the second component. Positive values of DeltaY indicate that Y increases as the value of X increases. Negative values of DeltaY indicate that Y decreases as the value of X increases.
etc.	etc.	etc.

Sample Applications

- Determining equilibrium (stability or formation) constants, Q_i , for the binding of a ligand or substrate to a mixture of metal ions, enzymes, or surface sites.
- Resolving equilibrium constants, Q_i , and rate constants, ΔY_i , for uncoupled (parallel) reactions from the dependence of the apparent rate constant on X.

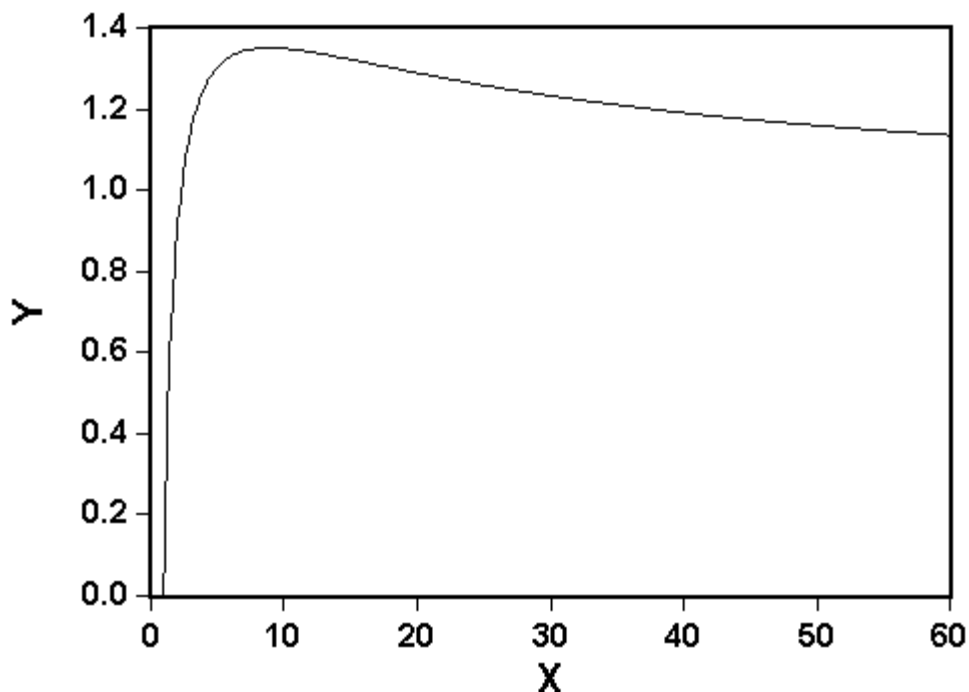
Remarks

At $X = 0$, $Y = 0$.

When automatic initial estimates are made, **FitAll** assumes that the data are sorted on column number 1; that is, the X-values.

FitAll will calculate initial estimates only when you are fitting the data to one saturation curve.

Ftn 0206: Uncoupled Saturation Curves (Non zero Origin)



Equation

The general form of the function is:

$$Y = P_1 + \sum_{i=1}^n \left(\frac{P_{2i} * P_{2i+1} * X}{[1 + P_{2i} * X]} \right)$$

in which

- Y is the measured response.
- X is the independent variable, often the time in seconds.
- n is the number of coupled saturation curves. "n" can have values from 1 to 10;

Ten variations of the function are available. For example:

- $$Y = P_1 + \frac{(P_2 * P_3 * X)}{(1 + P_2 * X)}$$

- $$Y = P_1 + \frac{(P_2 * P_3 * X)}{(1 + P_2 * X)} + \frac{(P_4 * P_5 * X)}{(1 + P_4 * X)}$$

Parameters

Parameter	Name	Comments
P1	Y ₀	Limiting value of Y when X is zero.
P2	Q1	Equilibrium quotient (formation constant) for the first component
P3	DeltaY1	Amplitude of Y for the first component. Positive values of DeltaY indicate that Y increases as the value of X increases. Negative values of DeltaY indicate that Y decreases as the value of X increases.
P4	Q2	Equilibrium quotient (formation constant) for the second component
P5	DeltaY2	Amplitude of Y for the second component. Positive values of DeltaY indicate that Y increases as the value of X increases. Negative values of DeltaY indicate that Y decreases as the value of X increases.
etc.	etc.	etc.

Sample Applications

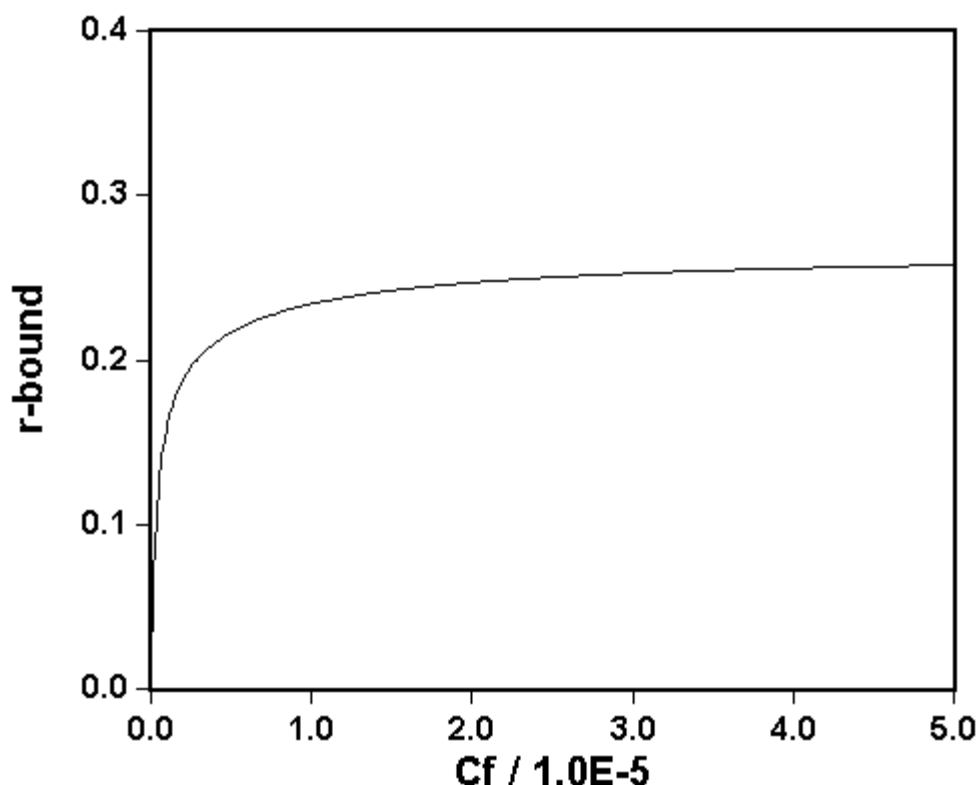
- Determining equilibrium (stability or formation) constants, Q_i , for the binding of a ligand or substrate to a mixture of metal ions, enzymes, or surface sites.
- Resolving equilibrium constants, Q_i , and rate constants, ΔY_i , for uncoupled (parallel) reactions from the dependence of the apparent rate constant on X.

Remarks

When automatic initial estimates are made, **FitAll** assumes that the data are sorted on column number 1; that is, the X-values.

FitAll will calculate initial estimates only when you are fitting the data to one saturation curve.

Ftn 0207: DNA-DRUG Binding: Multi-Site, Single Experiment; f's as parameters



Equation

The general form of the function is:

$$Y = \frac{1}{2} * \sum_{j=1}^n \left(\frac{|P_{2j-1}| * |P_{2j}| * X}{[1 + |P_{2j-1}| * X]} \right)$$

in which:

- n is the number of binding curves. It can have a value from 1 to 10.
- X is the (free) concentration of the binding agent (drug or antibiotic).
- Y is the binding ratio, rbound, in (moles drug bound)/(moles DNA base pairs). The divisor of two appears in the equation to convert from 'base pairs' to 'dinucleotides'.
- Pj, the parameters to be resolved, are constrained to values greater than or equal to zero. These are the only physically meaningful values.

There are ten variations of this function. For example:

$$Y = \frac{|P1| * |P2| * X}{2 * (1 + |P1| * X)}$$

$$Y = \frac{|P1| * |P2| * X}{2 * (1 + |P1| * X)} + \frac{|P3| * |P4| * X}{2 * (1 + |P3| * X)}$$

Parameters

Parameter	Name	Comments
P1	Q1	Equilibrium quotient (formation constant) for the first component or site type.
P2	DeltaY1	Amplitude of Y at high X for the first component or site type. The sum of the 'fractional site distributions', f, of binding sites with formation constant Q1.
P3	Q2	Equilibrium quotient (formation constant) for the second component or site type.
P4	DeltaY2	Amplitude of Y at high X for the second component or site type.
...
P19	Q10	Equilibrium quotient (formation constant) for the tenth component or site type.
P20	DeltaY10	Amplitude of Y at high X for the tenth component or site type.

Sample Applications

- Determining equilibrium (stability or formation constants) quotients, Qi, for the binding of an antibiotic to DNA.

Remarks

When X = 0, Y = 0.

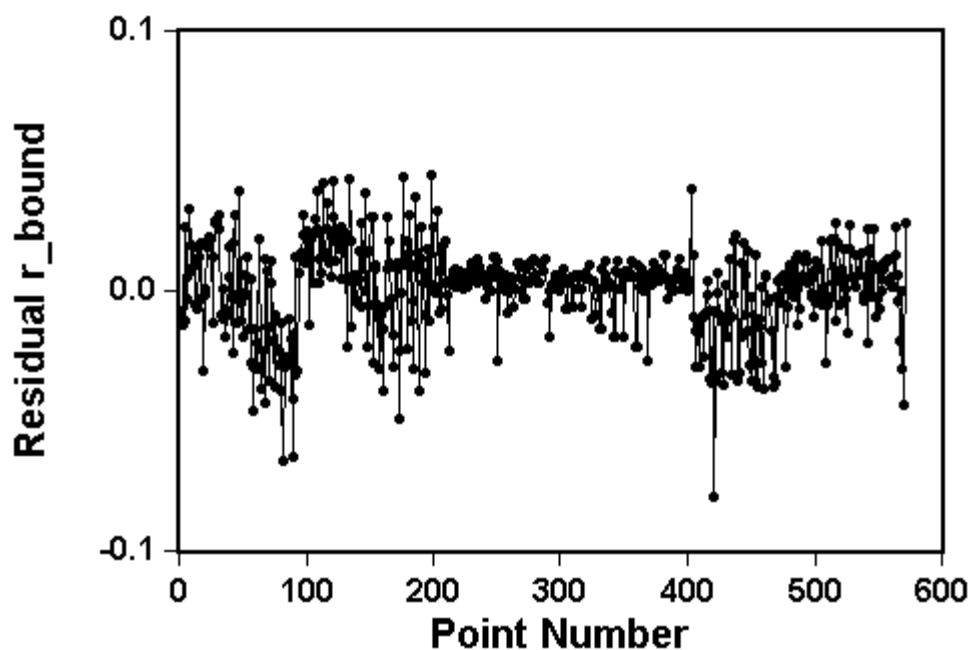
At high X, the limiting value of Y is 0.5.

The sum of all 'fractional site distributions' is *assumed* to be one.

When automatic initial estimates are made, **FitAll** assumes that the data are sorted on column number 1, that is, the X-values.

FitAll will calculate initial estimates only when you are fitting the data to one saturation curve.

Ftn 0208: DNA-DRUG Binding: Multi-Site, Multi-Experiment; f's as variables



Equation

The general form of the function is:

$$Y = \frac{1}{2} * \sum_{j=1}^n \left(\frac{A_j * |P_j| * X}{[1 + |P_j| * X]} \right)$$

in which

- n is the number of different or unique equilibrium quotients (formation constants). It can have a value from 1 to 10.
- A_j is the sum of the fractional site distributions, f_n, that are mapped to the parameter P_j.
- The 'fractional site distributions', (f_n, fraction of base pairs of site type n) that are stored in the data file, are treated as independent variables by this function, while they are treated as constants by [function 0209](#)^[24].
- X is the (free) concentration of the binding agent (drug or antibiotic).
- Y is the binding ratio, r_{bound}, in (moles drug bound)/(moles DNA base pairs).
- The divisor of two appears in the equation to convert from 'base pairs' to 'dinucleotides'.
- P_j, the parameters to be resolved, are constrained to values greater than or equal to zero (>= 0). These are the only physically meaningful values.

There are ten variations of this function. For example:

$$Y = \frac{A1 * |P1| * X}{2 * (1 + |P1| * X)}$$

$$Y = \frac{A1 * |P1| * X}{2 * (1 + |P1| * X)} + \frac{A2 * |P2| * X}{2 * (1 + |P2| * X)}$$

NOTE:
 This function assumes that the data set contains at least 13 columns.
 The data columns are assumed to be organized like this:

1. X, concentration of the drug.
2. f1, fraction of base pairs of site type 1.
3. f2, fraction of base pairs of site type 2.
-
11. f10, fraction of base pairs of site type 10.
12. (Optional) Experiment number (0 to 32000).
13. Y, binding ratio, rbound.
14. SigmaY, measurement error associated with Y.

Constants

Constant	Name	Comments
K1	f1 to Q	<p>Site type 1 to equilibrium quotient (formation constant) mapping constant.</p> <p>The value of K1 specifies the equilibrium quotient to which the fractional distribution of site type 1, f1, contributes.</p> <p>For example, if K1=1 then site 1 is assumed to have an equilibrium quotient equal to Q1. If K1=5, site 1 is assumed to have an equilibrium quotient equal to Q5.</p> <p>If the value of K1 is 0 or does not correspond to the index of one of the parameters (equilibrium quotients) being resolved, the fractional distribution for site 1, f1, will not contribute to the fit.</p> <p>Site type to equilibrium quotient mapping constants are assumed to be integers with values from 0 to 10.</p>

Constant	Name	Comments
		Default value is 1.
K2	f2 to Q	Site type 2 to equilibrium quotient (formation constant) mapping constant. Default value is 2
...
K10	f10 to Q	Site type 10 to equilibrium quotient (formation constant) mapping constant. Default value is 10.

Parameters

Parameter	Name	Comments
P1	Q1	Equilibrium quotient (formation constant) for the first component. The 'site types' (dinucleotides) that have this equilibrium quotient are specified by the 'mapping' constants K1 to K10. For example, if K1= 1 then site type 1 (with a fractional distribution of f1) has an equilibrium quotient equal to Q1 and if K5=1 then site type 5 also has an equilibrium quotient equal to Q1. Each equilibrium quotient must have at least one fractional site distribution mapped to it.
P2	Q2	Equilibrium quotient (formation constant) for the second component.
..
P10	Q10	Equilibrium quotient (formation constant) for the tenth component.

Sample Applications

- Determining equilibrium (stability or formation constants) quotients, Q_j , for the binding of an antibiotic to DNA.

Remarks

The 'fractional site distributions', f_n , that are stored in the data file, are treated as *independent variables* by

this function.

The sum of all 'fractional site distributions' for one data point ought to be one. That is, for one row of data, the sum of the values in columns 2 to 11 should be 1.0.

When setting up the function (Analyze, Setup) the 'Number of binding curves' corresponds to the number of unique (different or separable) equilibrium quotients (formation constants) that can be resolved.

Because this function has 11 independent variables, it is generally most useful in the 'fit graph' to plot Y versus the point number, since a plot of Y versus X1, the drug concentration, will not be a smooth curve.

A plot of the residuals versus point number will generally be the most useful visualization of the fit's quality.

If the data set contains multiple experiments, it may be helpful to sort it on the first column, the concentration, then on the experiment number, EN, before plotting the fit and residuals graphs.

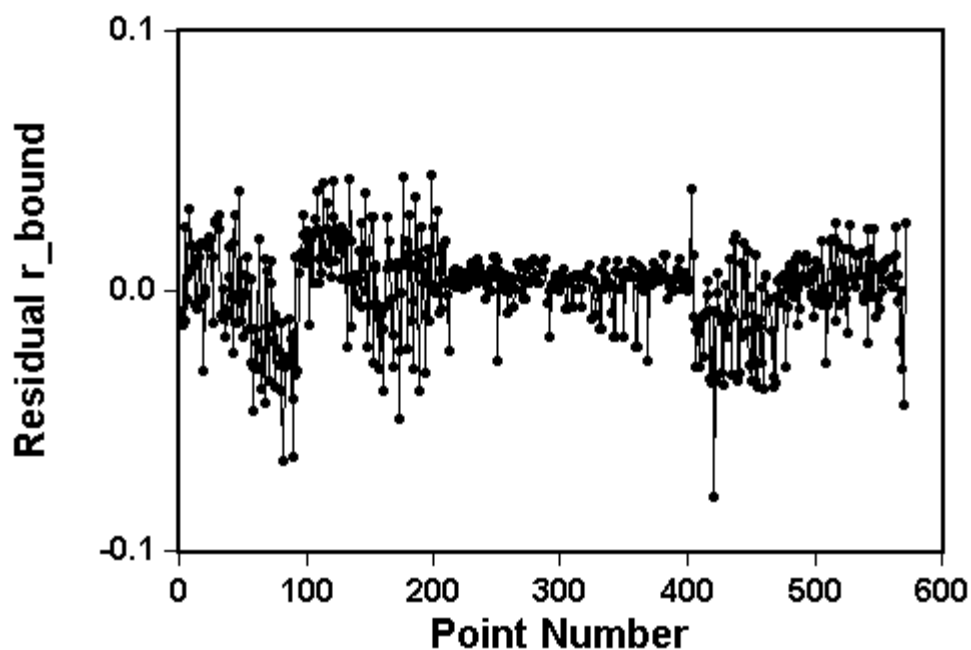
When $X = 0$, $Y = 0$.

At high X, the limiting value of Y is 0.5.

When automatic initial estimates are made, **FitAll** assumes that the data are sorted on column number 1, that is, the X-values.

FitAll will calculate initial estimates only when you are fitting the data to one saturation curve.

Ftn 0209: DNA-DRUG Binding: Multi-Site, Multi-Experiment; f's as constants



Equation

The general form of the function is:

$$Y = \frac{1}{2} * \sum_{j=1}^n \left(\frac{A_j * |P_j| * X}{[1 + |P_j| * X]} \right)$$

in which

- n is the number of different or unique equilibrium quotients (formation constants). It can have a value from 1 to 10.
- A_j is the sum of the fractional site distributions, f_n, that are mapped to the parameter P_j.
- The 'fractional site distributions', (f_n, fraction of base pairs of site type n) that are stored in the data file, are treated as constants by this function, while they are treated as independent variables by [function 0208](#)^[20].
- X is the (free) concentration of the binding agent (drug or antibiotic).
- Y is the binding ratio, r_{bound}, in (moles drug bound)/(moles DNA base pairs).
- The divisor of two appears in the equation to convert from 'base pairs' to 'dinucleotides'.
- P_j, the parameters to be resolved, are constrained to values greater than or equal to zero (>= 0). These are the only physically meaningful values.

There are ten variations of this function. For example:

$$Y = \frac{A1 * |P1| * X}{2 * (1 + |P1| * X)}$$

$$Y = \frac{A1 * |P1| * X}{2 * (1 + |P1| * X)} + \frac{A2 * |P2| * X}{2 * (1 + |P2| * X)}$$

NOTE:
 This function assumes that the data set contains at least 13 columns.
 The data columns are assumed to be organized like this:

1. X, concentration of the drug.
2. f1, fraction of base pairs of site type 1.
3. f2, fraction of base pairs of site type 2.
-
11. f10, fraction of base pairs of site type 10.
12. (Optional) Experiment number (0 to 32000).
13. Y, binding ratio, rbound.
14. SigmaY, measurement error associated with Y.

Constants

Constant	Name	Comments
K1	f1 to Q	<p>Site type 1 to equilibrium quotient (formation constant) mapping constant.</p> <p>The value of K1 specifies the equilibrium quotient to which the fractional distribution of site type 1, f1, contributes.</p> <p>For example, if K1=1 then site 1 is assumed to have an equilibrium quotient equal to Q1. If K1=5, site 1 is assumed to have an equilibrium quotient equal to Q5.</p> <p>If the value of K1 is 0 or does not correspond to the index of one of the parameters (equilibrium quotients) being resolved, the fractional distribution for site 1, f1, will not contribute to the fit.</p> <p>Site type to equilibrium quotient mapping constants are assumed to be integers with values from 0 to 10.</p>

Constant	Name	Comments
		Default value is 1.
K2	f2 to Q	Site type 2 to equilibrium quotient (formation constant) mapping constant. Default value is 2
...
K10	f10 to Q	Site type 10 to equilibrium quotient (formation constant) mapping constant. Default value is 10.

Parameters

Parameter	Name	Comments
P1	Q1	Equilibrium quotient (formation constant) for the first component. The 'site types' (dinucleotides) that have this equilibrium quotient are specified by the 'mapping' constants K1 to K10. For example, if K1= 1 then site type 1 (with a fractional distribution of f1) has an equilibrium quotient equal to Q1 and if K5=1 then site type 5 also has an equilibrium quotient equal to Q1. Each equilibrium quotient must have at least one fractional site distribution mapped to it.
P2	Q2	Equilibrium quotient (formation constant) for the second component.
..
P10	Q10	Equilibrium quotient (formation constant) for the tenth component.

Sample Applications

- Determining equilibrium (stability or formation constants) quotients, Q_j , for the binding of an antibiotic to DNA.

Remarks

The 'fractional site distributions', f_n , that are stored in the data file, are treated as *independent variables* by

this function.

The sum of all 'fractional site distributions' for one data point ought to be one. That is, for one row of data, the sum of the values in columns 2 to 11 should be 1.0.

When setting up the function (Analyze, Setup) the 'Number of binding curves' corresponds to the number of unique (different or separable) equilibrium quotients (formation constants) that can be resolved.

Because this function has 11 independent variables, it is generally most useful in the 'fit graph' to plot Y versus the point number, since a plot of Y versus X1, the drug concentration, will not be a smooth curve.

A plot of the residuals versus point number will generally be the most useful visualization of the fit's quality.

If the data set contains multiple experiments, it may be most useful to sort it on the first column, the concentration, then on the experiment number, EN, before plotting the fit and residuals graphs.

When $X = 0$, $Y = 0$.

At high X, the limiting value of Y is 0.5.

When automatic initial estimates are made, **FitAll** assumes that the data are sorted on column number 1, that is, the X-values.

FitAll will calculate initial estimates only when you are fitting the data to one saturation curve.

Appendix

[Getting Help](#) ³⁰

[Adding Functions to FitAll](#) ³¹

[Example Using Functions 0207/8/9](#) ³²

Getting Help

To get technical or other assistance from MTR Software you can:

Visit the FAQ page on MTR Software's web site at:

www.fitall.com

Email MTR Software at:

support@fitall.com

Write to MTR Software at:

MTR Software

77 Carlton Street, Suite 808

Toronto ON Canada

M5B 2J7

Telephone MTR Software at:

416-596-1499

Describe your problem or difficulty as completely as you can. We will try to answer your query quickly and completely.

You should also include your email address as well as your daytime, evening and weekend telephone numbers.

Adding Functions to FitAll

There are three ways to add your own specialized functions to *FitAll*.

1. You can contact **MTR Software** to get a quotation on the cost of creating a custom **FitAll Function Library** for you.
2. The *FitAll* Programmer's Guide, which is included with **FitAll Research Edition**, explains:
 - how to modify the supplied source code for the User Defined **FitAll Function Libraries** and
 - how to compile them using Borland Delphi version 5, 6, 7, 2005 and 2006.
3. You can contact **MTR Software** and request that the function be added to one of *FitAll*'s **Function Libraries**.

Example 1: Using Functions 0207/8/9

The purpose of this example is to illustrate one approach to use when fitting multiple sets of drug-dna binding data.

This example:

1. Uses several sets of actual binding data, although the names have been changed (to protect the innocent!).
2. Will demonstrate a procedure / process for fitting the data, but will not provide the actual final solution. -- That is left as an exercise!

Background

The binding of drugs, such as antibiotics, to DNA is important because the strength and/or specificity of the binding may be used as a measure of the likely effectiveness of the drug in the treatment of a disease.

NOTE:

Functions 0207 to 0209 assume that the equilibrium quotient (formation constant) for a given binding site is independent of the presence of other binding sites. That is, cooperative effects (synergies) are assumed to be absent.

If life were simple, a drug would bind to DNA with a single, measurable equilibrium quotient (formation constant) and that would be the end of the story. Unfortunately, or fortunately depending on your perspective, life isn't quite so simple.

DNA is composed of nucleotides and certain drugs bind to specific *pairs* of nucleotides. That is, drugs bind to *specific dinucleotides* with a specific equilibrium quotient (formation constant).

There are ten different dinucleotides to which a drug may bind.

The consequences of this are:

1. A drug may:
 - bind to more than one site type (dinucleotide).
 - bind to more than one site type (dinucleotide) with the same effectiveness (equilibrium quotient (formation constant)).
 - bind to more than one site type (dinucleotide) with differing effectiveness.
2. The effectiveness of a drug may be related to the specific DNA site type (dinucleotide) to which it binds; that is, to its specificity. This may not be the site to which it most strongly binds.
3. To resolve the situation for a given drug it is necessary to measure the equilibrium quotient (formation constant) of the drug with DNA samples that have different (fractional) distributions of dinucleotides (binding sites). This can be done by using DNA samples from different species or by synthesizing DNA with different dinucleotide (binding site) distributions for use in the binding experiments.

The **Binding Curves *FitAll* Function Library** includes sample data sets that can be used to test functions 0207, 0208 and 0209. The data sets are named F0207TST01.DTA to F0207TST08.DTA and F0208TST01_to_08.DTA.

The data sets F0207TST01.DTA to F0207TST08.DTA:

- Each contain data from a single binding experiment using the same drug, but a different type of DNA.
- Each contains the values of the fractional amount of each site type (dinucleotide) in the DNA that was used. These values are stored in data columns 2 to 11.

The data set F0208TST01_to_08.DTA was created by combining (appending) the eight data sets F0207TST01.DTA to F0207TST08.DTA into one so that all of the data can be analyzed at once using either function 0208 or 0209. Data column 12, EN, identifies the 'Experiment Number'. -- After combining the data, it was sorted on the first data column, Cf, then on the 'EN' column.

General Approach

The ultimate objective is:

- To analyze all of the data using function 0208 or 0209.
- To determine the drug's equilibrium quotient (formation constant) with each of the (DNA) site types (dinucleotides).

Here's how to do it:

1. Fit several (or all) of the single experiment data sets using function 0207.

The steps are:

- A. Plot the 'Data graph' and decide whether the data is likely to be described by one or more binding curves.
 - B. If you expect that one binding curve is likely to be sufficient, or if you are not sure:
 - i. Fit the data using function 0207 using one binding curve.
 - ii. Plot the fit and residuals graphs.
 - iii. If the residuals graph does not show any trends you can assume that one binding curve is all that can be resolved from the data. If, however, there are trends in the residuals graph, it is likely that more than one binding curve is required to adequately describe the data.
 - C. If the data is likely to be described by more than one binding curve do the following:
 - i. Fit the first part of the data to one binding curve.
 - ii. Fit a larger segment of the data to two binding curves using the results of the first fit to provide the initial estimates of the equilibrium quotient (formation constant) and amplitude for the first part of the curve and manually provide initial estimates for the second binding curve.
 - iii. Continue until all of the discernable binding curves have been fit and both the fit and residuals graphs indicate that a reasonably good fit has been obtained.
2. Compare the amplitudes of the of the binding curves obtained in 1 with the fractional site type values, in data columns 2 to 11. The objective is to arrive at an initial hypothesis (guess) as to which equilibrium quotient (formation constant) applies to which site type.

This is probably best done by creating several tables:

- one listing the results of the fits obtained using function 0207, for example,

Single Experiment Results Summary		
Experiment, EN	Q	DeltaY
1		
2		

Single Experiment Results Summary		
Experiment, EN	Q	DeltaY
3		
etc.		

- one that summarizes the results of the single experiment fits; that is, lists the number of different equilibrium quotients (formation constants) and their values, for example,

Proposed Separable Equilibrium Quotients	
Q Number	Approximate Value
1	
2	
3	
etc.	

- one listing the site type fractional distributions for all of the experiments, for example,

Fractional Site Type Distributions, f, by Experiment Number, EN											
EN	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10	Sum of f's
1	0.278	0.121	0.079	0.124	0.124	0.102	0.093	0.052	0.005	0.023	1.001
2											
3											
4											
5											
6											
7											
8											

- one listing the proposed site type to equilibrium quotient mappings, for example

Proposed Site Type to Equilibrium Quotient Mapping by Experiment Number, EN										
EN	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10
1										
2										
3										
4										
5										
6										
7										
8										

The objective is to assign one of the Q's to one or more site type until the sum of the fractional distributions approximately equals the amplitude (ΔY) of the corresponding Q.

Some "guess work" is required, especially at the initial stages, as initially there will not be sufficient information to make definitive assignments.

3. Repeat step 2 with a second data set and combine the results / site type to Q assignments.
4. Fit the data from all of the experiments using the results from the previous steps as a starting point to:
 - A. Assign initial estimates of the equilibrium quotients (formation constants), Q.
 - B. Assign values to the constants, K, to map the site types to the equilibrium quotients (formation constants), Q. The mapping constants can have values from 0 to 10. -- A mapping constant, K, of zero means that the drug does not bind to that site type.
 - C. Save the setup so that you don't have to be re-entered each time
5. Plot the residuals graph for the overall fit. This will help to identify the data set that is most problematic; that is, gives the worst fit and should be the next one considered for 'individual' attention.
6. Repeat steps 2 to 6 beginning with the data set identified in step 5 and combining its results with those obtained from the earlier analyses.
7. Repeat the above steps until the fit obtained in step 5 can not be further improved.

NOTE:
 If the number of experiments and / or the diversity of DNA samples is not sufficiently large, it may not be possible to arrive at a unique / definitive solution. If

this is the case, the results may be used to as a guide to designing additional experiments.

Fitting Single Experiment Data

In this stage, the data for the individual experiments are analyzed using function 0207.

The objectives are:

- To determine the number of binding curves that can be resolved from each experiment's data.
- To determine the equilibrium quotient (formation constant) and amplitude of each binding curve.

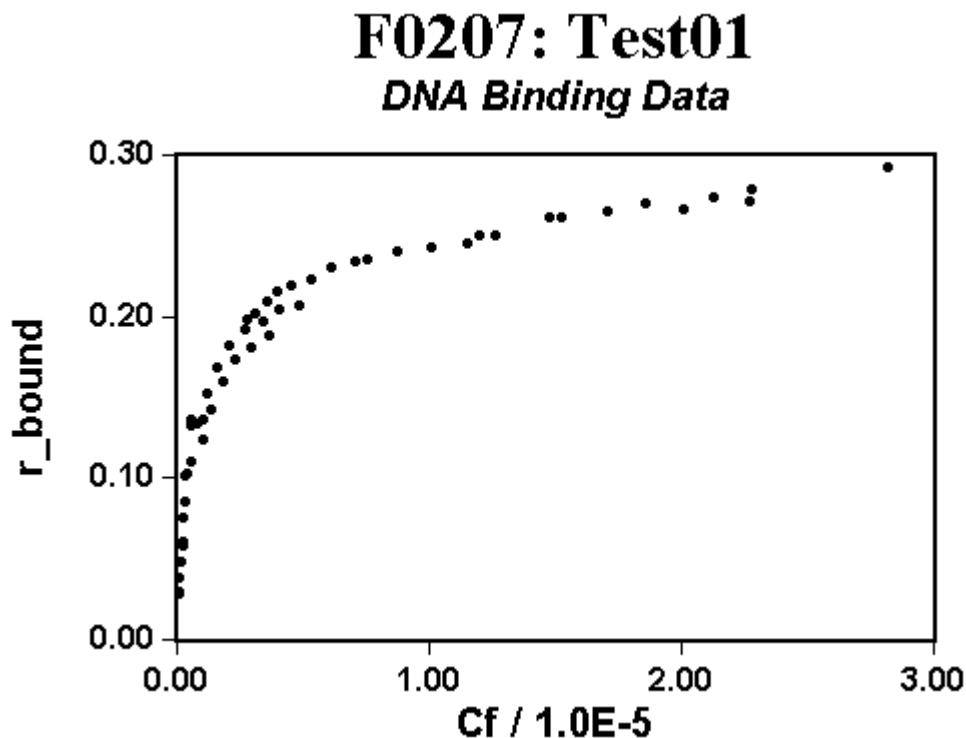
Fitting of a single experiment's data using function 0207 is illustrated in the sections [Fitting Experiment 1 Data](#)^[39] and [Fitting Experiment 2 Data](#)^[47].

Fitting the other sample single experiment data sets is left as an exercise; however, the results of their analyses are summarized in the [Stage 2](#)^[50] section.

Fitting Experiment 1 Data

In this section the data in the example f0207tst01.dta data set will be analyzed using function 0207.

The data graph looks like this:



1. It appears that more than one binding curve *may* be required. Since it is not absolutely clear that more than one binding curve will be needed, it is best to fit the entire data set to one curve and inspect the results.

To do this:

- Select Analyze, Setup or click the f_x button.
- In the 'Analyze Setup' dialog box that appears, click on the 'Save/Recall Setup' tab.
- In the pick list scroll down to and select the 'Test_f0207_1A' item.
- Click the Recall setup button.
- Click on the various tabs in the Analyze Setup dialog to see the initial settings that will be used in the analysis.
- Click the Ok button to accept the current setup.
- Select Analyze, Analyze or click the $f \rightarrow$ button to do the analysis.

The results of the analysis should look like this:

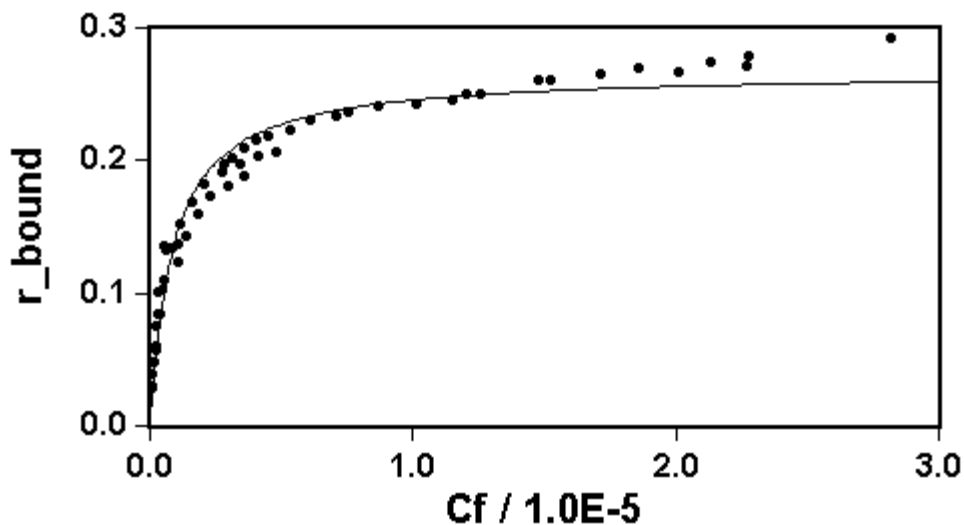
File:	c:\program files\fitall60\data\f0207tst01.dta
Title:	F0207: Test01
Sub-Title:	DNA Binding Data
Function:	0207..DNA-DRUG Binding: Multi-Site, Single Experiment; f's as parameters
Analysis Method:	Non-Linear Least Squares (nlls)
Analysis Range:	1 to 54 of 54
Weighted as:	1
Variance:	0.00023001073609074
Std. Dev. of Fit:	0.0151661048424024 after 6 iterations

Parameter	Name	Value	Std. Dev.
1	Q1	1.151E+0006	8.150E+0004
2	DeltaY1	5.339E-0001	8.492E-0003

The fit graph looks like this:

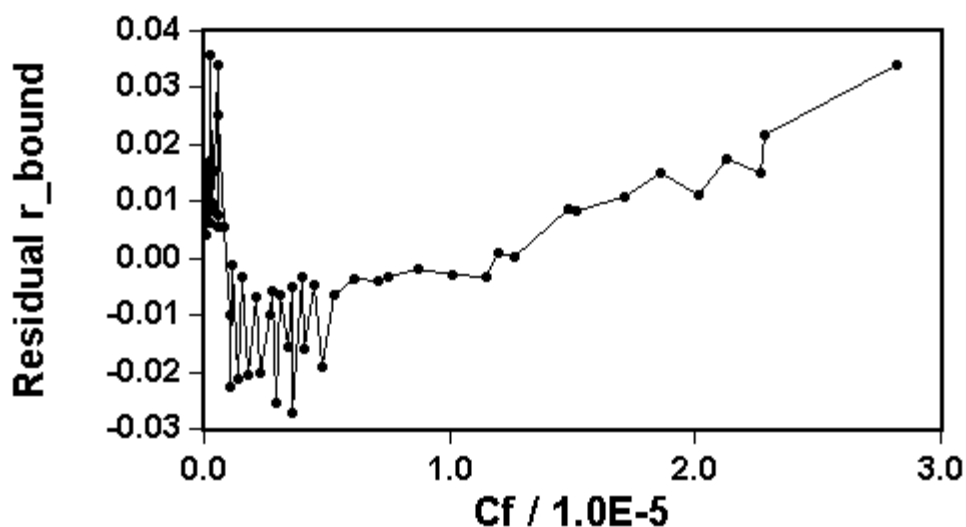
F0207: Test01

DNA Binding Data



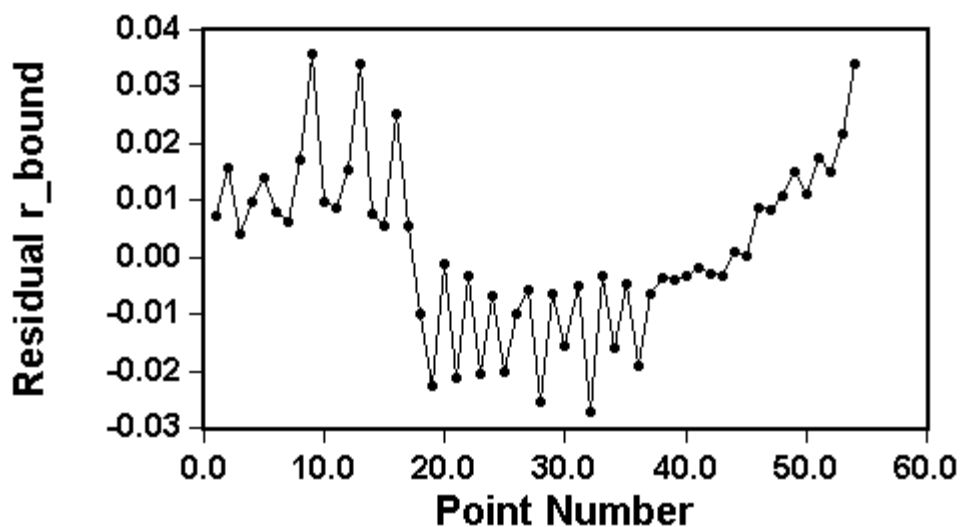
The residuals graph looks like this:

F0207: Test01 DNA Binding Data



To better see the trends in the residuals at low values of Cf change the bottom axis to a logarithmic scale or a 'point number' scale. When re-plotted it should look like this:

F0207: Test01 DNA Binding Data



The trends visible in the fit and residuals graphs clearly indicate that more than one binding curve is required to fit this data.

2. To get a preliminary value for the first equilibrium quotient, fit the first 20 data points to function 0207 with one binding curve.

To do this:

- Select Analyze, Setup or click the f_x button.
- In the 'Analyze Setup' dialog box that appears, click on the 'Save/Recall Setup' tab.
- In the pick list scroll to and select the 'Test_f0207_1B' item.
- Click the Recall setup button. This will retrieve the (predefined) setup that can be used to analyze the first 20 points of the data to one binding curve.
- Click the OK button.
- To do the analysis, Select Analyze, Analyze or click the $f \rightarrow$ button.

The results of the analysis should look like this:

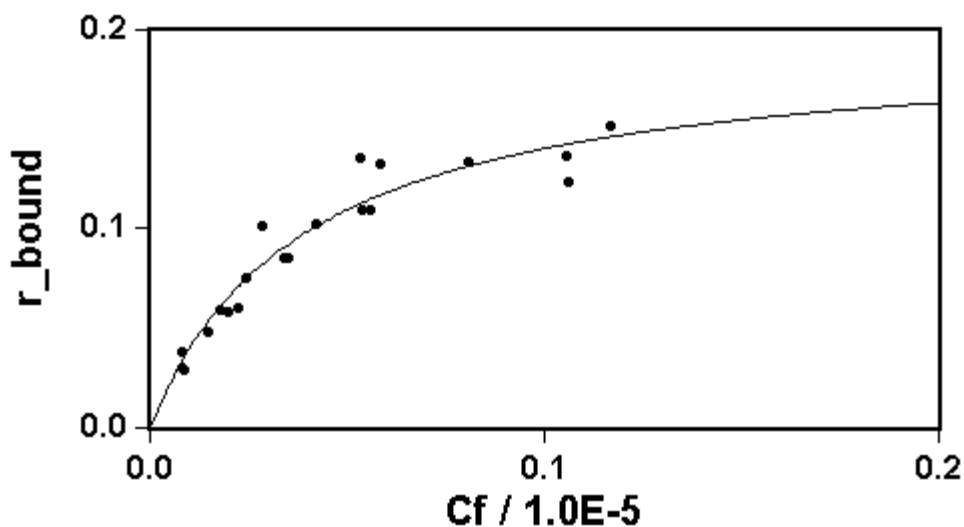
File:	c:\program files\fitall60\data\f0207tst01.dta
Title:	F0207: Test01
Sub-Title:	DNA Binding Data
Function:	0207..DNA-DRUG Binding: Multi-Site, Single Experiment; f's as parameters
Analysis Method:	Non-Linear Least Squares (nlls)
Analysis Range:	1 to 20 of 54
Weighted as:	1
Variance:	0.000110138327390675
Std. Dev. of Fit:	0.0104946809094262 after 6 iterations

Parameter	Name	Value	Std. Dev.
1	Q1	2.555E+0006	4.286E+0005
2	DeltaY1	3.906E-0001	2.858E-0002

The fit graph looks like this:

F0207: Test01

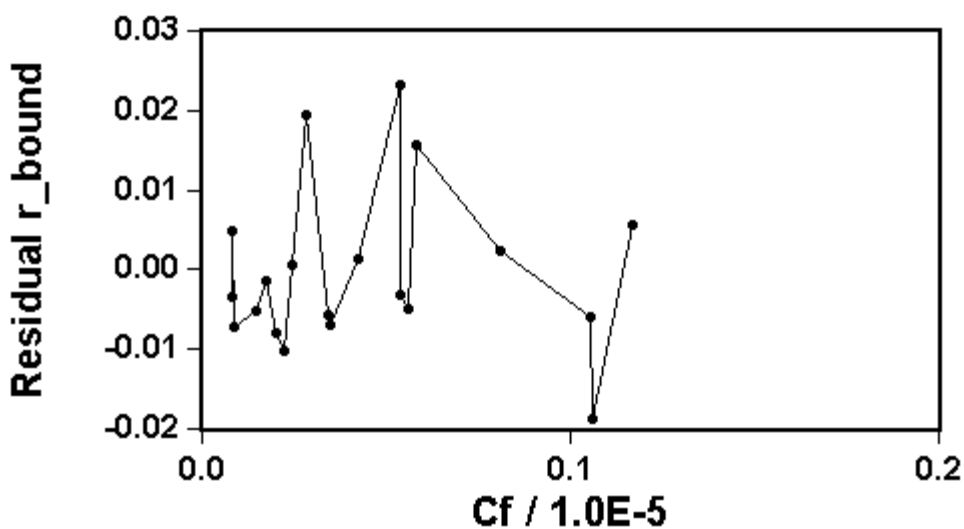
DNA Binding Data



The residuals graph looks like this:

F0207: Test01

DNA Binding Data



3. To fit all of the data in experiment 1 to two equilibrium quotients using function 0207 do this:
 - Select Analyze, Setup or click the f_x button.
 - In the 'Analyze Setup' dialog box that appears, click on the 'Save/Recall Setup' tab.
 - In the pick list scroll to and select the 'Test_f0207_1C' item.

- Click the Recall setup button. This will retrieve the (predefined) setup that can be used to analyze the data to two binding curves.

NOTE:
 The values of Q1 and DeltaY1 are those obtained from the analysis of the first 20 data points.

 The initial (estimated) values of Q2 and DeltaY2 were arrived at by inspection of the data graph and were manually entered.

- Click the OK button.
- To do the analysis, select Analyze, Analyze or click the $f \rightarrow$ button.

The results of the analysis should look like this:

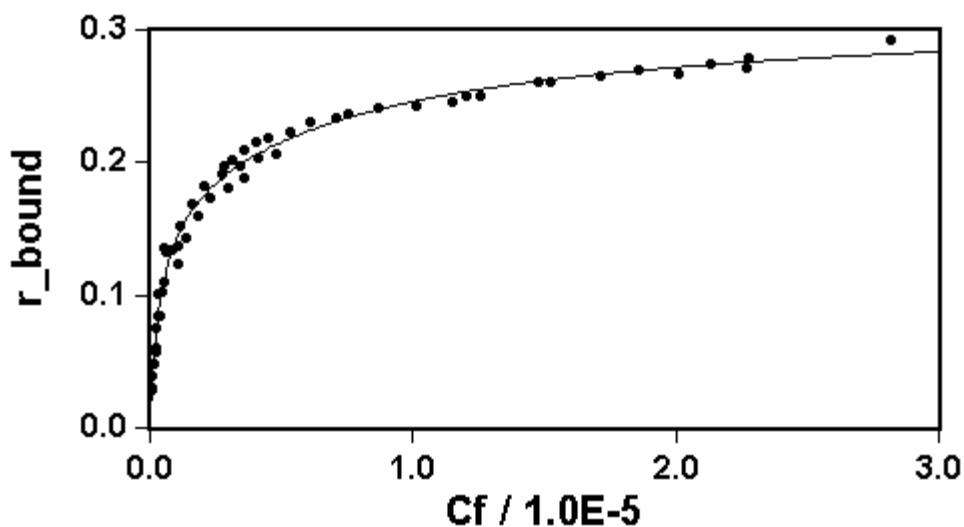
File:	c:\program files\fitall60\data\f0207tst01.dta
Title:	F0207: Test01
Sub-Title:	DNA Binding Data
Function:	0207..DNA-DRUG Binding: Multi-Site, Single Experiment; f's as parameters
Analysis Method:	Non-Linear Least Squares (nlls)
Analysis Range:	1 to 54 of 54
Weighted as:	1
Variance:	7.27034137447744E-5
Std. Dev. of Fit:	0.00852662968263395 after 4 iterations

Parameter	Name	Value	Std. Dev.
1	Q1	2.912E+0006	5.398E+0005
2	DeltaY1	3.353E-0001	3.831E-0002
3	Q2	1.317E+0005	5.322E+0004
4	DeltaY2	2.952E-0001	2.459E-0002

The fit graph looks like this:

F0207: Test01

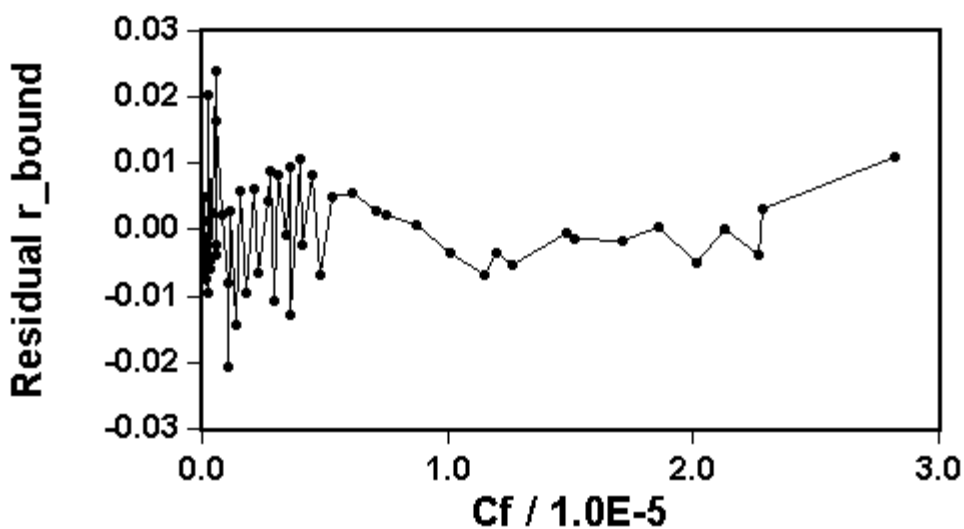
DNA Binding Data



The residuals graph looks like this:

F0207: Test01

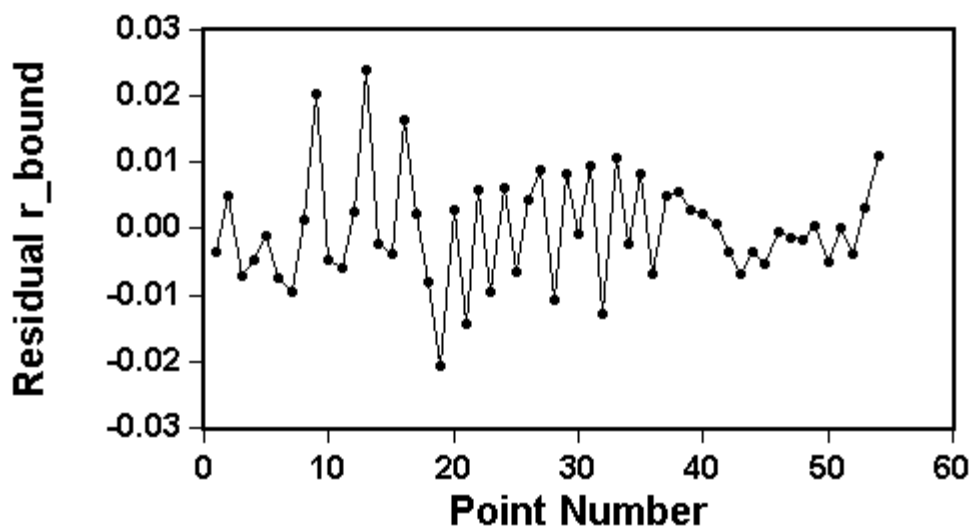
DNA Binding Data



When the bottom axis is changed to 'Point Number', the residuals graph looks like this:

F0207: Test01

DNA Binding Data



Inspection of the fit, the fit graph and the residual graph shows that:

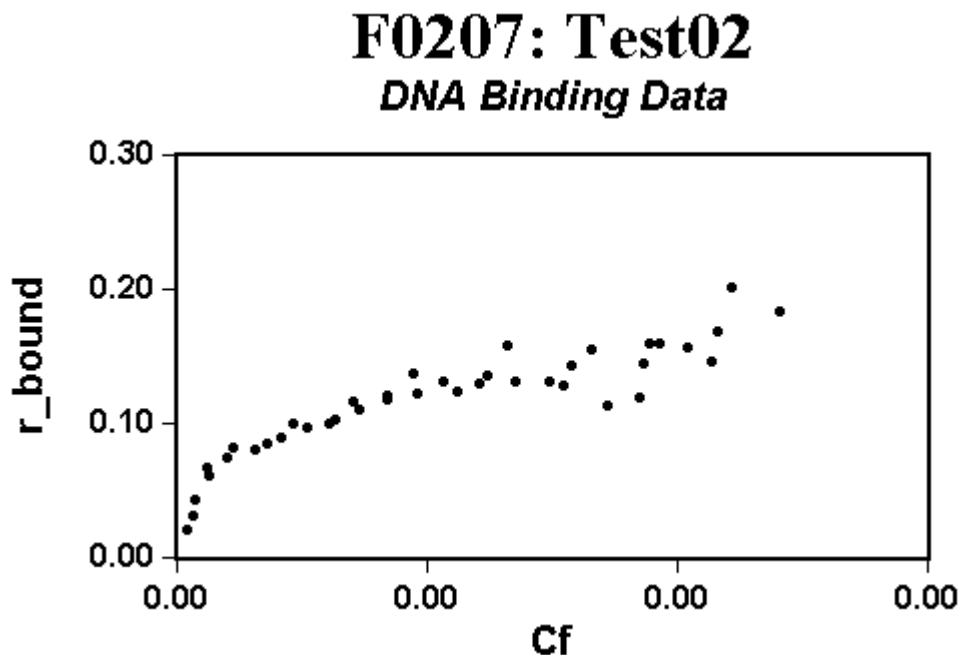
- The standard deviation of the fit is smaller (0.0085) than when only one binding curve was used (0.015).
- More importantly, there are no significant trends visible / discernible in either the fit graph or the residuals graph.

At this point, it is reasonable to conclude that the experiment 1 data (in the data file f0207tst01.dta) can be adequately described by two binding curves with equilibrium quotients (formation constants) of approximately 2.9×10^6 and 1.3×10^5 .

Fitting Experiment 2 Data

In this section the data in the example f0207tst02.dta data set will be analyzed using function 0207.

The data graph looks like this:



Fit the entire data set to one curve like this:

- Select Analyze, Setup or click the f_x button.
- In the 'Analyze Setup' dialog box that appears, click on the 'Save/Recall Setup' tab.
- In the pick list scroll down to and select the 'Test_f0207_2A' item.
- Click the Recall setup button.
- Click on the various tabs in the Analyze Setup dialog to see the initial settings that will be used in the analysis.
- Click the Ok button to accept the current setup.
- Select Analyze, Analyze or click the $f \rightarrow$ button to do the analysis.

The results of the analysis should look like this:

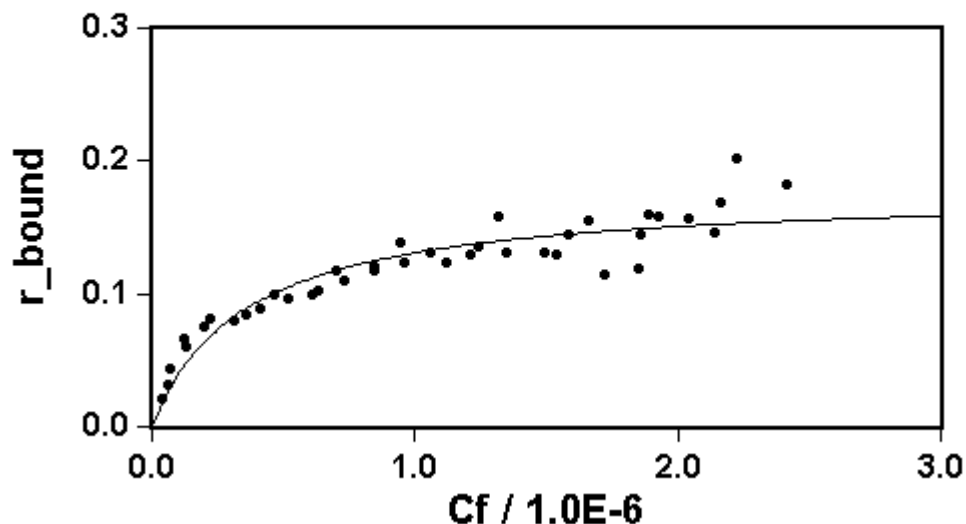
File:	c:\program files\fitall60\data\f0207tst02.dta
Title:	F0207: Test02
Sub-Title:	DNA Binding Data

Function:	0207..DNA-DRUG Binding: Multi-Site, Single Experiment; f's as parameters
Analysis Method:	Non-Linear Least Squares (nlls)
Analysis Range:	1 to 40 of 40
Weighted as:	1
Variance:	0.000229323083967078
Std. Dev. of Fit:	0.0151434171826268 after 8 iterations

Parameter	Name	Value	Std. Dev.
1	Q1	2.803E+0006	4.674E+0005
2	DeltaY1	3.551E-0001	1.571E-0002

The fit graph looks like this:

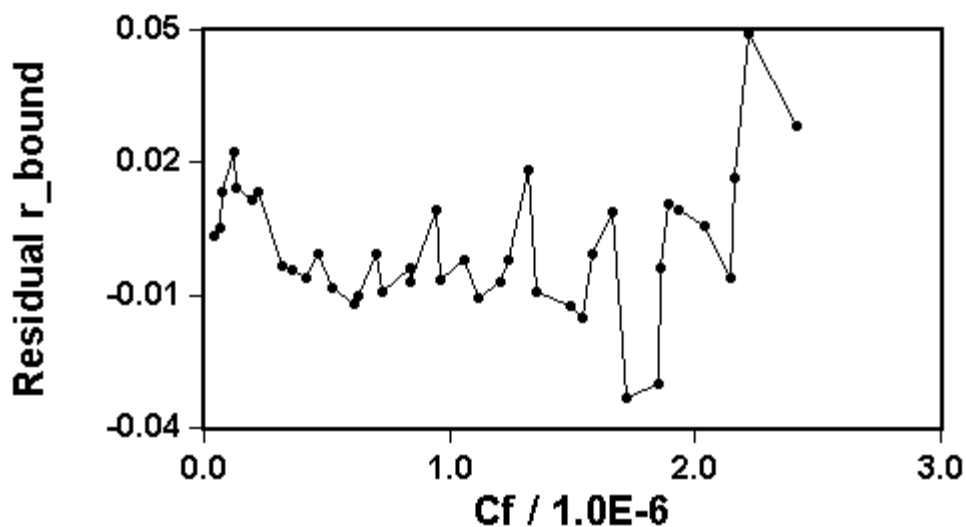
F0207: Test02 DNA Binding Data



The residuals graph looks like this:

F0207: Test02

DNA Binding Data



Inspection of the fit graph and the residual graphs shows that there are no significant trends.

NOTE:

Because the residuals graph shows a very slight trend, an attempt was made to fit the data to two binding curves.

The result of the analysis was that the standard deviation for the second equilibrium quotient (formation constant) was larger than its own value! -- In other words, it was not statistically significant.

At this point, it is reasonable to conclude that the experiment 2 data (in the data file f0207tst02.dta) can be adequately described by one binding curve with an equilibrium quotient (formation constant) of approximately $2.8e6$.

Results: Single Experiment Fits

The results of the single experiment fits and the site type distributions are given in the following tables.

Single Experiment Results		
Experiment Number, EN	Q	DeltaY
1	<i>2.9e6</i>	0.335
1	<u>1.3e5</u>	0.295
2	<i>2.8e6</i>	0.355
3	4.9e6	0.387
4	5.0e6	0.358
5	4.9e6	0.434
6	<u>3.6e6</u>	0.399
7	<i>2.6e6</i>	0.577
8	<i>2.2e6</i>	0.478

The results of the single experiment fits suggest that there are two and possibly three or four different binding constants (formation constants). In the above table the 'different' formation constants are distinguished by their text format; namely, italic, underlined, bold and italic underlined.

The following table lists the initial proposed (separable) equilibrium quotients (formation constants) that *may* be resolvable. The experiment number, EN, column identifies the data set(s) that implied the equilibrium quotient's value.

Proposed (Separable) Equilibrium Quotients		
Q Number	Approximate Value	EN
1	5.0e6	3, 4, 5
2	1.3e5	1
3	2.5e6	1, 2, 7, 8
4	3.6e6	6

NOTE:

The third and fourth proposed equilibrium quotients, with approximate values of $2.5e6$ and $3.6e6$, may actually be an "average" of Q1 and Q2. For this reason, they are listed last and will be the last to be considered when fitting all of the data with function 0208 or 0209.

The site type distributions are summarized in the following table.

Fractional Site Type Distributions, f, by Experiment Number, EN											
EN	f1	f2	f3	f4	f5	f6	f7	f8	f9	f10	Sum of f's
1	0.278	0.121	0.079	0.124	0.124	0.102	0.093	0.052	0.005	0.023	1.001
2	0.194	0.081	0.103	0.141	0.067	0.118	0.148	0.097	0.010	0.043	1.002
3	0.184	0.072	0.062	0.145	0.062	0.113	0.155	0.102	0.011	0.052	0.958
4	0.157	0.073	0.124	0.144	0.068	0.124	0.152	0.136	0.017	0.040	1.000
5	0.151	0.071	0.106	0.106	0.049	0.114	0.141	0.112	0.069	0.079	0.998
6	0.153	0.073	0.109	0.109	0.050	0.121	0.145	0.114	0.059	0.071	1.004
7	0.037	0.023	0.111	0.097	0.012	0.128	0.106	0.222	0.141	0.122	0.999
8	0.174	0.075	0.110	0.136	0.052	0.133	0.154	0.108	0.017	0.040	0.999

The last column, the "Sum of f's" column, has been included to provide a cross check of the distribution values. The sum of the site type distributions for one experiment's data should be 1.0.

This is true for all but one of the experiments.

For experiment number 3, the sum is 0.958. This deviation, although not large, may be due to a legitimate experimental error or a typographical error. When fitting all of the experiments' data to function 0208 or 0209 this deviation should be kept in mind.

Fitting All the Data

Before fitting all of the data to function 0208 or 0209 it is necessary to decide on a mapping among the site types and the equilibrium quotients (formation constants).

For the sample data that is supplied with **FitAll** this is somewhat problematic as one site type's fractional distribution does not dominate in any of the experiments.

Consequently, it will be necessary to make a "best guess" and see where that leads.

The following table lists the first of several "best guesses".

	Site Type to Equilibrium Quotient Mapping										
Attempt	f1 to Q#	f2 to Q#	f3 to Q#	f4 to Q#	f5 to Q#	f6 to Q#	f7 to Q#	f8 to Q#	f9 to Q#	f10 to Q#	Std. Dev. of Fit
A	2	1	1	1	2	2	1	1	1	1	
B											
C											
D											
E											
F											

Fitting All the Data: A

In this section all of the binding data will be fit using the first "best guess" of the site type to equilibrium quotient (formation constant) mapping given in the following table as "Attempt A".

	Site Type to Equilibrium Quotient Mapping										
Attempt	f1 to Q#	f2 to Q#	f3 to Q#	f4 to Q#	f5 to Q#	f6 to Q#	f7 to Q#	f8 to Q#	f9 to Q#	f10 to Q#	Std. Dev. of Fit
A	2	1	1	1	2	2	1	1	1	1	
B											
C											
D											
E											
F											

To do the fit, do the following:

- Retrieve / Open the data set named f0208tst01_to_08.dta by selecting the menu items File, Open.
- Fit the entire data set to function 0208 with two equilibrium quotients (formation constants) like this:
 - Select Analyze, Setup or click the f_x button.
 - In the 'Analyze Setup' dialog box that appears, click on the 'Save/Recall Setup' tab.
 - In the pick list scroll down to and select the 'Test_f0208_A' item.
 - Click the Recall setup button.
 - Click on the various tabs in the Analyze Setup dialog to see the initial settings that will be used in the analysis.
 - Click the Ok button to accept the current setup.
 - Select Analyze, Analyze or click the $f \rightarrow$ button to do the analysis.
- The results of the analysis should look like this:

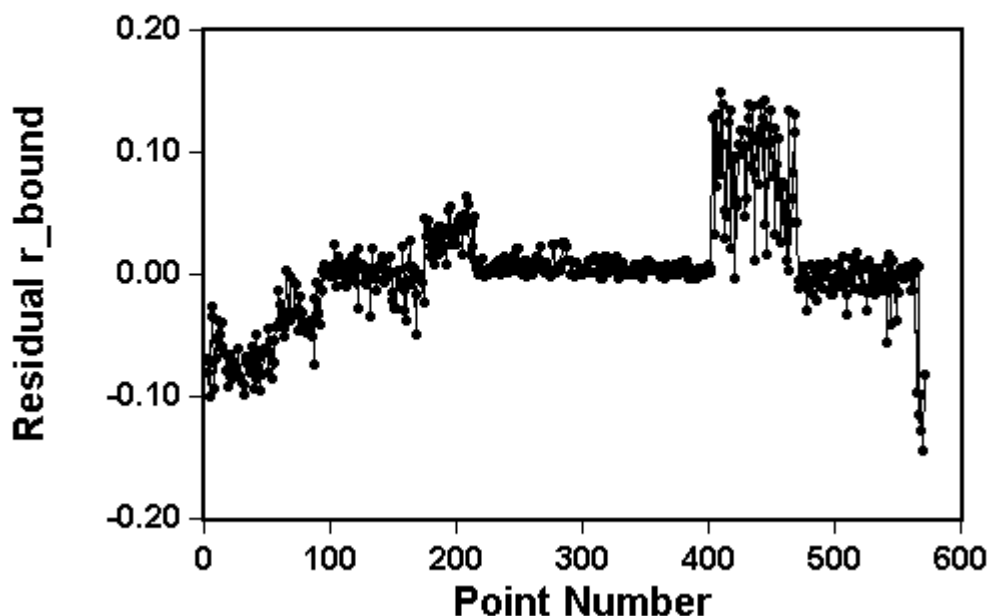
File:	c:\program files\fitall60\data\f0208tst01_to_08.dta
Title:	F0208: Test01-08: Multi-Experiment
Sub-Title:	DNA Binding Data

Function:	0208..DNA-DRUG Binding: Multi-Site, Multi-Experiment; f's as variables
Analysis Method:	Non-Linear Least Squares (nlls)
Analysis Range:	1 to 571 of 571
Weighted as:	1
Variance:	0.00198877265965285
Std. Dev. of Fit:	0.0445956574080128 after 7 iterations

Parameter	Name	Value	Std. Dev.
1	Q1	4.328E+0004	3.623E+0003
2	Q2	4.173E+0006	2.870E+0005
Constant			
1	f1 to Q	2.000E+0000	
2	f2 to Q	1.000E+0000	
3	f3 to Q	1.000E+0000	
4	f4 to Q	1.000E+0000	
5	f5 to Q	2.000E+0000	
6	f6 to Q	2.000E+0000	
7	f7 to Q	1.000E+0000	
8	f8 to Q	1.000E+0000	
9	f9 to Q	1.000E+0000	
10	f10 to Q	1.000E+0000	

- Since data from several experiments is being analyzed, the best visualization of the fit is given by the residuals graph. It should look like this:

F0208: Test01-08: Multi-Experin DNA Binding Data



- Clearly, there are significant trends / deviations in the residuals graph. The largest deviations are between point numbers 410 and 450. Inspection of the data (in the data window) indicates that these data points correspond to experiment number 7, consequently, it would be advisable to look at the single experiment results for experiment 7 when making the next "best guess" of the site type to equilibrium quotient assignments.

Fitting All the Data: B

In this section all of the binding data will be fit using the second "best guess" of the site type to equilibrium quotient (formation constant) mapping given in the following table as "Attempt B".

	Site Type to Equilibrium Quotient Mapping										
Attempt	f1 to Q#	f2 to Q#	f3 to Q#	f4 to Q#	f5 to Q#	f6 to Q#	f7 to Q#	f8 to Q#	f9 to Q#	f10 to Q#	Std. Dev. of Fit
A											
B	2	1	2	1	2	2	1	1	1	1	
C											
D											
E											
F											

To do the fit, do the following:

1. Retrieve / Open the data set named f0208tst01_to_08.dta by selecting the menu items File, Open.
2. Fit the entire data set to function 0208 with two equilibrium quotients (formation constants) like this:
 - Select Analyze, Setup or click the f_x button.
 - In the 'Analyze Setup' dialog box that appears, click on the 'Save/Recall Setup' tab.
 - In the pick list scroll down to and select the 'Test_f0208_B' item.
 - Click the Recall setup button.
 - Click on the various tabs in the Analyze Setup dialog to see the initial settings that will be used in the analysis.
 - Click the Ok button to accept the current setup.
 - Select Analyze, Analyze or click the $f \rightarrow$ button to do the analysis.
3. The results of the analysis should look like this:

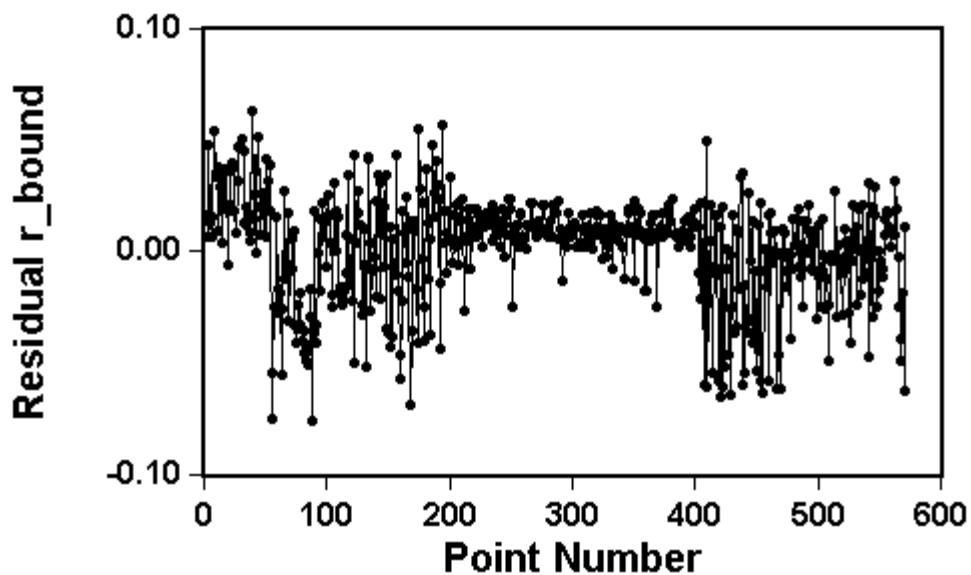
File:	c:\program files\fitall60\data\f0208tst01_to_08.dta
Title:	F0208: Test01-08: Multi-Experiment
Sub-Title:	DNA Binding Data
Function:	0208..DNA-DRUG Binding: Multi-Site, Multi-Experiment; f's as variables
Analysis Method:	Non-Linear Least Squares (nlls)
Analysis Range:	1 to 571 of 571
Weighted as:	1
Variance:	0.000564624682542452
Std. Dev. of Fit:	0.0237618324744211 after 7 iterations

Parameter	Name	Value	Std. Dev.
1	Q1	1.527E+0006	3.181E+0004
2	Q2	3.461E+0003	9.856E+0002

Parameter	Name	Value	Std. Dev.
Constant			
1	f1 to Q	2.000E+0000	
2	f2 to Q	1.000E+0000	
3	f3 to Q	2.000E+0000	
4	f4 to Q	1.000E+0000	
5	f5 to Q	2.000E+0000	
6	f6 to Q	2.000E+0000	
7	f7 to Q	1.000E+0000	
8	f8 to Q	1.000E+0000	
9	f9 to Q	1.000E+0000	
10	f10 to Q	1.000E+0000	

4. Since data from several experiments is being analyzed, the best visualization of the fit is given by the residuals graph. It should look like this:

F0208: Test01-08: Multi-Experin DNA Binding Data



- The standard deviation of this fit (0.02376) is smaller than that in the previous fit (0.04459) and the residuals graph shows fewer and smaller trends. This means that the site type to equilibrium quotient mapping used in this fit is more appropriate than that used in the previous fit.

Results: Fitting All the Data

The following table provides a summary of the seven (A to F) fits done by **MTR Software** to all of the data.

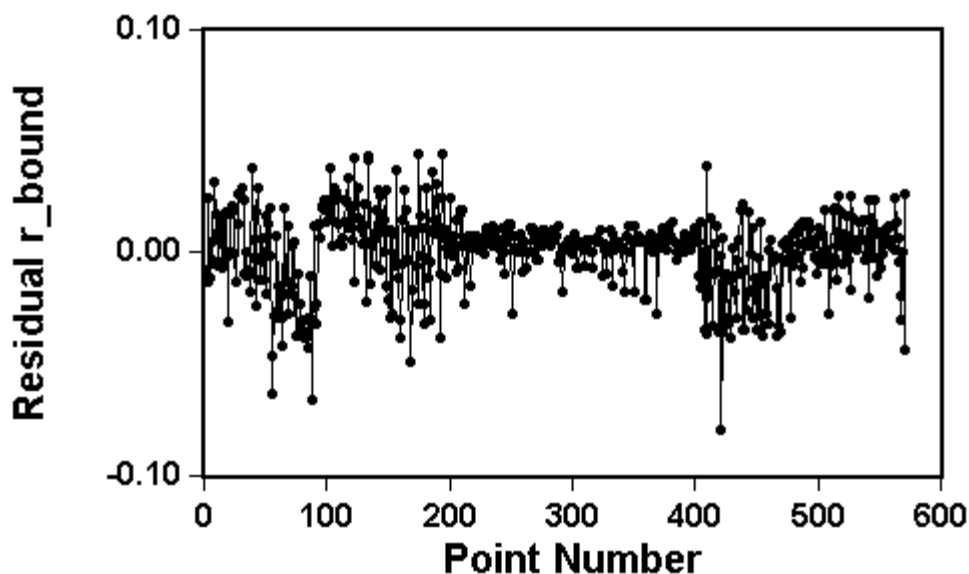
	Site Type to Equilibrium Quotient Mapping										
Attempt	f1 to Q#	f2 to Q#	f3 to Q#	f4 to Q#	f5 to Q#	f6 to Q#	f7 to Q#	f8 to Q#	f9 to Q#	f10 to Q#	Std. Dev. of Fit
A	2	1	1	1	2	2	1	1	1	1	0.04459
B	2	1	2	1	2	2	1	1	1	1	0.02376
C	2	1	2	1	2	2	2	1	1	1	0.01834
D	1	2	2	1	2	2	2	1	1	1	0.01735
E	1	2	1	2	2	2	2	1	1	1	0.01696
F	1	2	1	2	2	2	1	2	1	1	0.02036

The site type to equilibrium quotient mapping in Attempt E provides the best fit (smallest Std. Dev. of Fit) of this set of attempted / trial mappings.

Although Attempt E gives the best fit, it should be noted that:

- Attempt E's "Std. Dev. of Fit" is not all that much smaller than that obtained in Attempt D.
- The residuals graph for Attempt E shows significant trends, which can be seen in the following graph.

F0208: Test01-08: Multi-Experin DNA Binding Data



3. The fit obtained in Attempt E can **not** be the best possible fit and its site type to equilibrium quotient mapping is not the best possible estimate of site type equilibrium quotients (formation constants) for the drug that was used in these experiments.

More definitive results can be obtained by doing one or more of the following:

1. Make additional fits with the currently available data using different site type to equilibrium quotient mappings. (Note: There are more than a thousand possible variations).
2. Consider cases in which three or four equilibrium quotients are used.
3. Do additional experiments with DNA samples that have widely different site type distributions. It would be especially useful to have DNA samples that are dominated by one site type.

Index

- A -

Adding Functions to FitAll 31

Adsorption
Langmuir 4

- C -

Contacting MTR Software 30

Cooperative
saturation curve 12

- D -

DNA-DRUG Binding
example fit 32
Multi-Experiment; f's as constants 24
Multi-Site; Multi-Experiment; f's as variables
20
single experiment 18

- E -

Example
DNA-DRUG Binding 32
fitting all the data A 53
fitting all the data B 55
fitting all the data summary 58

- F -

Function 4, 6, 9, 12, 14, 16, 18, 20, 24
adding to FitAll 31
cooperative saturation curve 12
DNA-DRUG Binding 18, 20, 24
Langmuir Adsorption 4
saturation curve 6, 9
uncoupled saturation curve 14, 16
Function Reference 3

- H -

Help 30
How to
contact MTR Software 30

- L -

Langmuir Adsorption 4

- S -

Saturation Curve
cooperative 12
coupled 6, 9
Langmuir 4
uncoupled 14, 16