Submitted by Civilized Software Inc.

Step-Wise Multiple-Site Binding

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Cooperative effects often arise when there are multiple binding sites for a ligand that are located spatially close to one another. Some of the issues that arise are discussed in "Mixed and uniform cooperativity of ligand binding to multisite proteins: The cooperativity types allowed by the Adair equation and conditions for them" by Edward P. Whitehead, in the Journal of Theoretical Biology, pp. 153:170, vol. 87, 1980.

A particular situation of interest is that where many F molecules bind step-wise through a series of reactions to a G molecule with distinct affinities. For example, oxygen binds to hemaglobin in this manner. This is one explanation for apparent cooperative binding. Let G_0 denote free G. Thus we may consider the situation:

$F + G_0 \underset{D_1}{\overset{A_1}{\rightleftharpoons}} G_1,\ F + G_1 \underset{D_2}{\overset{A_2}{\rightleftharpoons}} G_2, \ldots, F + G_{N-1} \underset{D_N}{\overset{A_N}{\rightleftharpoons}} G_N.$

When N (the number of F-binding sites on each G molecule) is not large, the kinetic model differential equations can be used in curve-fitting; however, in general, we deal with the equilibrium model instead.

Define the molar equilibrium constants $K_i = A_i/D_i$. Let $F(t)$ be the concentration of (unbound) F at time t, let $G_i(t)$ be the concentration of G_i at time t, and let F_e and G_{ie} be these concentrations at $t = t_e$, the time when our system approaches equilibrium. Then: $K_i = G_{ie}/(G_{i-1,e}F_e)$, so $K_1K_2 \ldots K_i = G_{ie}/(G_{0e}F_e^i)$. Now we may define $B_i = K_1K_2 \ldots K_i$.

Now, let F_b be the concentration of bound F molecules at equilibrium, so $F_b = G_{1e} + 2G_{2e} + \ldots + NG_{Ne}$. Note that $F_b + F_e$ is the total concentration of F present, *i.e.* $F_b + F_e = F(0)$. Also, let H be the concentration of G molecules in either a bound or free state, so $H = G_{0e} + G_{1e} + \ldots + G_{Ne}$.

Now, define v as the mean number of F molecules bound to each G molecule. Then $v = F_b/H$, or
$v = (G_{1e} + 2G_{2e} + \ldots + NG_{Ne})/(G_{0e} + G_{1e} + \ldots + G_{Ne})$.
But, $G_{ie} = B_iG_{0e}F_e^i$, so
$v = (B_1G_{0e}F_e + 2B_2G_{0e}F_e^2 + \ldots + NB_NG_{0e}F_e^N)/$
$(G_{0e} + B_1G_{0e}F_e + B_2G_{0e}F_e^2 + \ldots + B_NG_{0e}F_e^N)$, or
$v = (B_1F_e + 2B_2F_e^2 + \ldots + NB_NF_e^N)/$
$(1 + B_1F_e + B_2F_e^2 + \ldots + B_NF_e^N)$.

This is the Adair-Klotz stepwise equilibrium model.

Now given data points (F_e, F_b), each based on different initial values of H and $F_e + F_b$, corresponding data points of the form $(F_e, F_b/H)$ can be constructed, and v thus allowing the parameters $B_1, B_2, \ldots, B_N$ to be estimated (which hence provides estimates of the equilibrium constants $K_1, K_2, \ldots, K_N$). If the number of sites, N, is not known, N can be set to 1, 2, 3, etc., and that value of N which yields the best fit can be taken as the estimate of the true N-value. Note the model for data points of the form (F_e, G_{0e}) can be expressed in terms of a *ROOT* expression in $G_0(0)$ and $K_1, \ldots, K_N$. Note also that data of the form $(F_e, F_b/H)$ has error in both the first and second components. This means that, at a minimum, correct weights should be used in fitting.

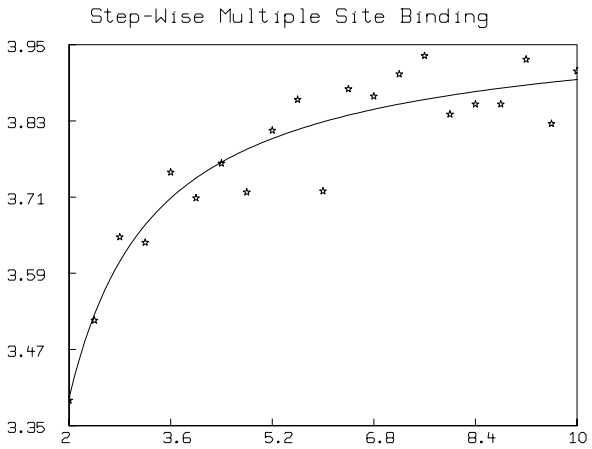
The following is a *MLAB* dialog that demonstrates the above mentioned curve fitting for $N = 4$. We first define the model function $v(F_e)$, read-in the data, set the initial guesses for B_1, B_2, B_3 and B_4, and then fit the data to the model.

```
fct p(x) = 1+sum(I,1,4, B[I]*x^I)
fct v(fe) = fe*p'x(fe)/p(fe) /* model function */
data = read(msb, 21, 2)
t = minv((data col 1)):maxv(data col 1)!100
B = 2:5 /* initial guesses */

fit(B), v to data with weight ewt(data)

final parameter values
   value      error      dependency parameter
 2.7807664  164.6641428  0.9999816   B[1]
 0.0702266   16.0436904  0.9992536   B[2]
 3.1455369  133.7340925  0.9999952   B[3]
 2.7828358  116.4894630  0.9999972   B[4]
3 iterations
best weighted sum of squares = 3.047449e+01
weighted root mean square error = 1.338886e+00
weighted deviation fraction = 9.451356e-03
R squared = 8.959082e-01

draw data lt none pt star ptsize 0.01
draw points(v,t)
top title "Step-Wise Multiple Site Binding"
view
```



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