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 hemoglobin in this manner. This is one explanation for apparent cooperative binding. Let \( G_0 \) denote free G. Thus we may consider the situation:

\[
F + G_0 \xrightarrow{A_1/D_1} G_1, F + G_1 \xrightarrow{A_2/D_2} G_2, \ldots, F + G_{N-1} \xrightarrow{A_N/D_N} G_N.
\]

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 (bound) \( F \) 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:

\[
K_i = \frac{G_{ie}}{(G_{i-1}eF_e)}, \quad K_1K_2 \ldots K_i = \frac{G_{ie}/(G_0eF_e^i)}{G_{i-1}eF_e},
\]

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

\[
v = \frac{(G_{1e} + 2G_{2e} + \ldots + NG_{Ne})}{(G_{0e} + G_{1e} + \ldots + G_{Ne})}.
\]

But, \( G_{ie} = B_iG_0eF_e^i \), so

\[
v = \frac{(B_1G_0eF_e + B_2G_0eF_e^2 + \ldots + NB_NG_0eF_e^N)}{(B_1G_0eF_e + B_2G_0eF_e^2 + \ldots + B_NG_0eF_e^N)},
\]

or

\[
v = \frac{(B_1F_e + B_2F_e^2 + \ldots + NB_NF_e^N)}{(1 + B_1F_e + B_2F_e^2 + \ldots + B_NF_e^N)}.
\]

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 \( v \)

\[
\text{fit(B), } v \text{ to data with weight w}(\text{data})
\]

final parameter values
value error dependency parameter
2.7807664 0.164641428 0.0999816 B[1]
0.0702266 0.160396904 0.9992636 B[2]
3.1455369 0.133740925 0.9999952 B[3]
2.7828358 0.116449630 0.9999972 B[4]
3 iterations

R squared = 8.95902e-01

draw data 1t none pt star ptsize 0.01
draw points(v,t)
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