

Second-Order Binding

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Consider the reaction $F + G \xrightleftharpoons[K_2]{K_1} B$.

The study of this reaction is common in chemistry and biochemistry. For example, F could be a hormone or drug and G the associated receptor sites. The symbol B represents the bound complex.

Let us also allow $F(t)$, $G(t)$, and $B(t)$ to be functions which specify the concentrations of F , G , and B respectively at time t . Then we have the differential equation model:

$$\begin{aligned} dB/dt(t) &= K_1 F(t)G(t) - K_2 B(t), & B(0) &= B_0, \\ F(t) &= F_0 - (B(t) - B_0), \\ G(t) &= G_0 - (B(t) - B_0), \end{aligned}$$

where F_0 , G_0 , and B_0 are the initial concentrations of F , G , and B respectively, and the molar association and dissociation rate constants K_1 and K_2 appear as the proportionality constants for the terms which occur in dB/dt .

Note that $K_1 = (dB/dt(0) + K_2 B_0)/(F_0 G_0)$, so K_1 may be estimated from the initial velocity $dB/dt(0)$, which can, in turn, be estimated from a few points.

The solution to our differential equation is:

$$B(t) = (S(B_0 - R) - R(B_0 - S)e^{d \cdot K_1 \cdot t}) / (B_0 - R - (B_0 - S)e^{d \cdot K_1 \cdot t}),$$

where

$$\begin{aligned} S &= A + d/2 \\ R &= A - d/2 \\ d &= 2(A^2 - (F_0 + B_0)(G_0 + B_0))^{1/2} \\ A &= (F_0 + G_0 + 2B_0 + K_2/K_1)/2 \end{aligned}$$

An appropriate definition of this function in MLAB involves using auxiliary functions as follows:

```
fct B(T) = H1((F0+G0+2*B0+K2/K1)/2,T)
fct H1(A,T) = H2(A,SQRT(4*(A^2-(F0+B0)*(G0+B0))),T)
fct H2(A,D,T) = H3(A+D/2,A-D/2,EXP(D*K1*T))
fct H3(S,R,E) = ((B0-R)*S-(B0-S)*R*E)/(B0-R-(B0-S)*E)
```

It is useful to study this example carefully. Many occasions will arise where functions will need to be expressed with sub-functions in a similar manner.

Suppose we have specific kinetic data, time versus B -concentration, appearing as the rows of a two-column matrix, BM . Note time vs. G -concentration or time vs. F -concentration can be easily converted to time vs. B -concentration. In MLAB, if GM is a 2 column matrix of time vs. G -concentration for example, we merely type

```
* BM = (GM COL 1)&'(G0-B0-(GM COL 2))
```

and BM is then the desired time vs. B -concentration matrix of data points.

We may use the curve-fitting facility of MLAB to compute estimates of K_1 and K_2 , and even F_0 , G_0 , and/or B_0 if necessary.

The equilibrium constant, K , of the reaction, is defined as $K = K_1/K_2$. At equilibrium (say at time t_e) we have $dB/dt(t_e) = 0$, and hence $K_1F(t_e)G(t_e) - K_2B(t_e) = 0$.

Thus, $K = B(t_e)/(F(t_e)G(t_e)) = B(t_e)/((F_0 + B_0 - B(t_e))(G_0 + B_0 - B(t_e)))$.

Define $B_e(F_0, G_0, B_0) = B(t_e)$, the amount of B at equilibrium or “saturation”. Then solving for $B_e(F_0, G_0, B_0)$, we have the following equation, called the *saturation equation*.

$$B_e(F_0, G_0, B_0) = [(F_0+G_0+2B_0+1/K) - ((F_0+G_0+2B_0+1/K)^2 - 4(F_0+B_0)(G_0+B_0))^{1/2}]/2.$$

If we have data points for a so-called saturation curve consisting of pairs of F_0 values with associated B_e values for fixed values of G_0 and B_0 , then curve-fitting can be used with the above function in order to estimate K . Indeed K can be estimated even when we have points (F_0, G_0, B_e) from the saturation surface in 3-space, where arbitrary values of F_0 and G_0 have been paired, and B_0 is fixed.

Define $B_e = B(t_e)$, $F_e = F(t_e)$, and $G_e = G(t_e)$. Then from the basic relations: $K = B_e/(F_eG_e)$, $F_e + B_e = F_0 + B_0$, and $G_e + B_e = G_0 + B_0$, we may write a number of equivalent relationships.

Michaelis-Menten Equation(1) (B_e vs. F_e):

$$B_e = K(G_0 + B_0)F_e/(1 + KF_e)$$

Michaelis-Menten Equation(2) ($B_e/(B_0 + G_0)$ vs. F_e):

$$B_e/(B_0 + G_0) = KF_e/(1 + KF_e)$$

Lineweaver-Burk Equation ($1/B_e$ vs. $1/F_e$):

$$1/B_e = (1/(K(G_0 + B_0)))(1/F_e) + 1/(G_0 + B_0)$$

Eadie-Wilkinson-Dixon Equation (F_e/B_e vs. F_e):

$$F_e/B_e = F_e/(G_0 + B_0) + 1/(K(G_0 + B_0))$$

Scatchard Equation (B_e/F_e vs. B_e):

$$B_e/F_e = -KB_e + K(G_0 + B_0)$$

Hill Equation ($\log[(B_e/(G_0 + B_0))/(1 - B_e/(G_0 + B_0))]$ vs. $\log F_e$):

$$\log((B_e/(G_0 + B_0))/(1 - B_e/(G_0 + B_0))) = \log F_e + \log K$$

(Also see the “direct linear plot” of Cornish-Bowden in *Biochem. J.* Vol 137, p. 143, 1974)

Some of these relationships are inspired by analogous relations for enzyme reactions where they arise in different forms. Most are linear relations in K or $1/K$ for simple binding, and this accounts for their popularity—they are easy to use as models with linear regression methods in order to estimate K . For the non-linear Michealis-Menten forms, constraints are often necessary. *In spite of their traditional use, however, the errors introduced when transforming data to the appropriate form may limit the accuracy obtainable when using any of these models; and in general biased estimates of K can result.* See Rodbard, D., “Mathematics of Hormone-Receptor Interaction”, in *Receptors for Reproductive Hormones*, Plenum Pub. Corp.

The saturation equation above may thus be preferred, although usually there is little difference. At any rate, the values of K obtained using various models should be checked by computing theoretical predicted values for B_e vs. F_0 and comparing them to the observed values. The major difficulty for the various linear forms is that both the independent and the dependent-variable data values have non-normally-distributed error. As a result, linear Euclidean curve-fitting (with appropriate weights) should be employed with

these models. The results should be checked in the saturation equation. That value of K which yields the lowest sum-of-squares in the saturation model should be used.

Here is an example comparing the saturation equation model with the Michaelis-Menten (1) model and the Scatchard model.

```

FCT B(F0)=((F0+G0+2*B0+1/K)-SQRT((F0+G0+2*B0+1/K)^2-4*(F0+B0)*(G0+B0)))/2
FCT BE(FE) = (G0+B0)*FE/(1/K+FE)
FCT BS(BE) = -K*BE+K*(G0+B0)

B0 = 0; G0 = 1;

/* M = F0 values, BE values */
M = read(data,100,2)

/* generate M1 = corresponding data (FE,BE) for the Michaelis-Menten model */
M1 = (M COL 1) - (M COL 2) + B0
M1 COL 2 = M COL 2

/* generate M2 = corresponding data (BE/FE,BE) for the Scatchard model */
M2 = (M COL 2)
M2 COL 2 = (M1 COL 2) / (M1 COL 1)

K = 2
FIT(K), B TO M
final parameter values
      value          error          dependency    parameter
      1.996602727    0.0420400256          0          K
1 iterations
CONVERGED
best weighted sum of squares = 1.446128e-02
weighted root mean square error = 1.925622e-02
weighted deviation fraction = 1.849765e-02
R squared = 9.691570e-01
KS = K

FIT(K), BE TO M1
final parameter values
      value          error          dependency    parameter
      2.0043749954    0.0397621839          0          K

```

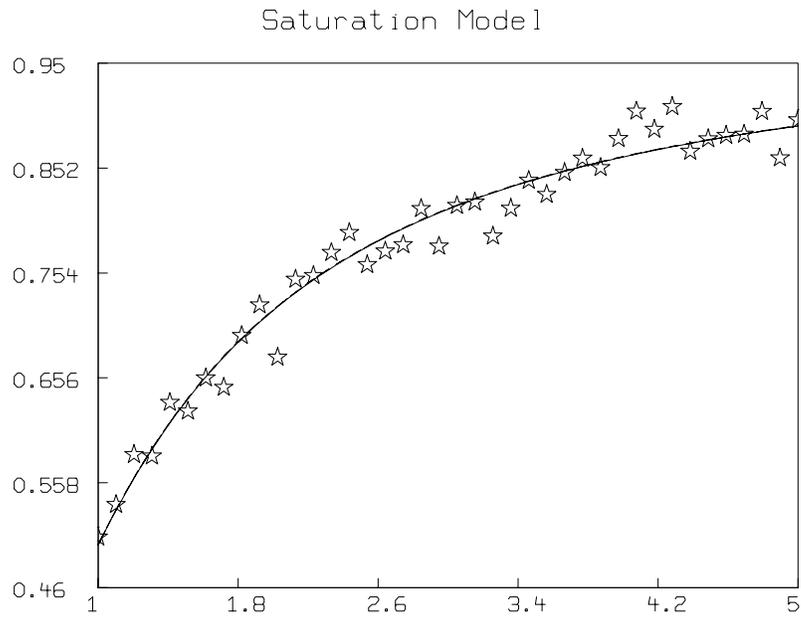
```
1 iterations
CONVERGED
best weighted sum of squares = 1.769429e-02
weighted root mean square error = 2.130023e-02
weighted deviation fraction = 2.026365e-02
R squared = 9.622617e-01
KM = K
```

```
FIT(K), BS TO M2
final parameter values
```

value	error	dependency	parameter
2.0008431423	0.0360417006	0	K

```
1 iterations
CONVERGED
best weighted sum of squares = 1.242737e-01
weighted root mean square error = 5.644913e-02
weighted deviation fraction = 8.426590e-02
R squared = 9.358569e-01
KC = K
```

```
/* draw MM model + data with the above K */
K = KS
TOP TITLE "Saturation Model"
DRAW M, LT NONE, PT STAR
DRAW POINTS(B, 1:5!101)
K = KM
DRAW POINTS(B, 1:5!101) LT DASHED
K = KC
DRAW POINTS(B, 1:5!101) LT ALTERNATE
VIEW
```

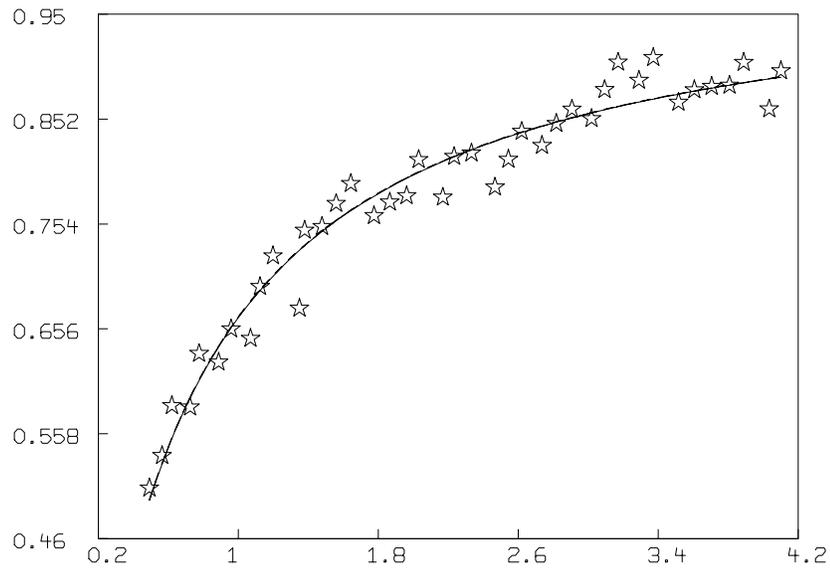


```

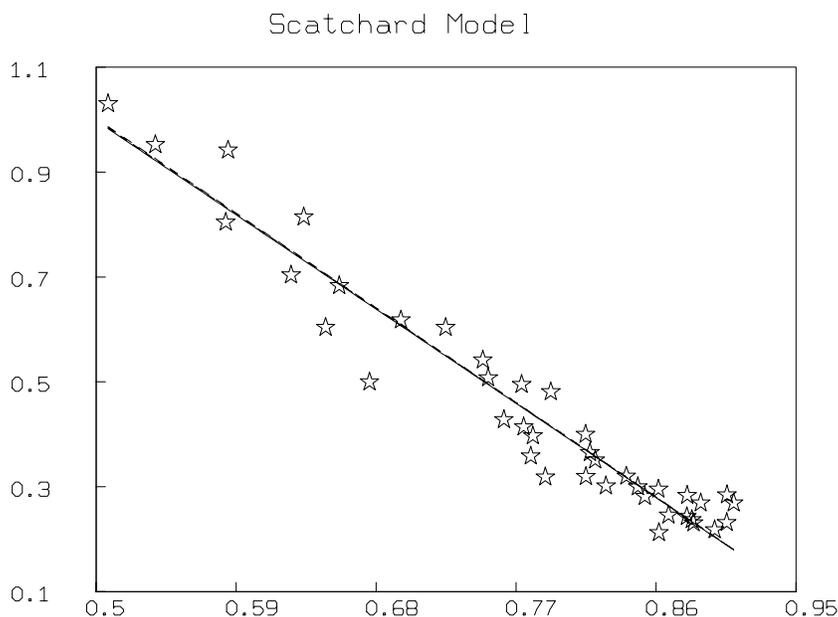
K = KS
TOP TITLE "Michaelis-Menten Model"
DRAW M1, LT NONE, PT STAR
DRAW POINTS(BE, MINV(M1 col 1):MAXV(M1 col 1)!101)
K = KM
DRAW POINTS(BE, MINV(M1 col 1):MAXV(M1 col 1)!101) LT DASHED
K = KC
DRAW POINTS(BE, MINV(M1 col 1):MAXV(M1 col 1)!101) LT ALTERNATE
VIEW

```

Michaelis-Menten Model



```
/* draw Scatchard model + data with the above K */  
K = KS  
TOP TITLE "Scatchard Model"  
DRAW M2, LT NONE, PT STAR  
DRAW POINTS(BS, MINV(M2 col 1):MAXV(M2 col 1)!101)  
K = KM  
DRAW POINTS(BS, MINV(M2 col 1):MAXV(M2 col 1)!101) LT DASHED  
K = KC  
DRAW POINTS(BS, MINV(M2 col 1):MAXV(M2 col 1)!101) LT ALTERNATE  
VIEW
```



Note that for the data studied above, all the models give comparable results. Indeed the estimated K -values are so close, the curves are almost superimposed.

The second Michaelis-Menten equation is useful when the amount, $G_0 + B_0$, is not known. Then measuring a quantity proportional to $B_e/(G_0 + B_0)$ vs. F_e is commonly done, and K can be determined in unknown units. Indeed, by introducing another parameter, D , to obtain $B_e/(B_0 + G_0) = DKF_e/(1 + KF_e)$ and computing D and K by fitting this model to data points $(F_e, B_e/(B_0 + G_0))$, where F_e and B_e are measured in moles and $B_0 + G_0$ is measured in grams, then $D(B_0 + G_0)$ has the unit moles, and so $(B_0 + G_0)/(D(B_0 + G_0)) = 1/D$ is the molecular weight of a G molecule. (This device for computing molecular weight assumes that $B_0 = 0$ or that an F molecule is much lighter than a G molecule.) Fitting the saturation equation to obtain both K and $G_0 + B_0$ may be a better approach.

Cooperative Binding

Often the binding of F and G is complicated by cooperative effects. Namely, K_1 and/or K_2 appear to be dependent upon the relative amount of B . This can be due to allosteric shape changes in the molecules or sites G which occur during binding. Various other explanations, including multiple classes of sites, are also possible. If K_1/K_2 increases as B increases, we have

positive cooperativity; if K_1/K_2 decreases as B increases we have negative cooperativity.

Suppose, then, that K_1 and K_2 are functions of B . Thus,

$$dB/dt(t) = K_1(B(t)) \cdot F(t) \cdot G(t) - K_2(B(t)) \cdot B(t),$$

with $F(t) = F_0 - (B(t) - B_0)$, $G(t) = G_0 - (B(t) - B_0)$, and $B(0) = B_0$, as before.

Now suppose that all cooperative effects are due to changes in K_2 , in particular, suppose

$$K_1(B) = K_1^0, \quad \text{and} \quad K_2(B) = K_2^0(1 + p \cdot B/(G_0 + B_0)).$$

This is the same as saying $K_2(B) - K_2(0) = pB/(G_0 + B_0)$, thus we assume that the change in K_2 from the “ground state” $K_2(0) = K_2^0$, is proportional to the fraction of occupied sites, $B/(G_0+B_0)$, with the proportionality-constant p .

Note then, that $dK_2/dt(B(t)) = (p/(G_0+B_0))dB/dt(t)$, so $dK_2/dB(B) = p/(G_0 + B_0)$.

There are of course many other functional relationships which could be postulated. For example, we could assume that $dK_2/dt(B(t)) = A(dB/dt)h$, or we could assume K_1 and K_2 vary together in certain ways. Indeed, changes in K_1 will give cooperative effects unobtainable by changes in K_2 alone. K_1 and K_2 need not change monotonically; we may have variation which results in intervals of positive cooperativity and other intervals of negative cooperativity.

Since cooperativity, without qualification as to its cause, is merely a mathematical description, and not a structural description, the choice of how $K_1(B)$ and $K_2(B)$ are defined is dependent upon the actual physical situation and the desired uses of the mathematical model. The particular choice here has the same effect as that made by DeMeyts in his analysis of cooperativity (DeMeyts, P., Woebroeck, M., “The structural basis of insulin-receptor binding and cooperative interactions”, in Membrane Proteins (ed. P.Nicholls et al.) FEBS 11th Meeting, Vol. 45 Symposium A4, Pergamon Press, pp. 319–323, 1977).

Now let t_e be the time when equilibrium is approached, and let $B(t_e) = B_e$, $F(t_e) = F_e$, and $G(t_e) = G_e$. Then, at equilibrium, we have the equilibrium constant K as a function of B_e , $K(B_e) = K_1(B_e)/K_2(B_e) = B_e/(F_e G_e)$. Thus, $K(B_e) = K_1^0/(K_2^0(1 + pB_e/(G_0 + B_0)))$, or, $K(B_e) = K_0/(1 + pB_e/(G_0 + B_0))$, where $K_0 = K(0)$.

Note for $-1 < p < 0$, we have positive cooperativity, for $p = 0$, we have no cooperativity ($K(B_e) = K(0)$), and for $p > 0$, we have negative cooperativity.

It is convenient to define p in terms of another parameter, a , called the F , G interaction factor, so that $p = (1 - a)/a$, and hence $a = 1/(1 + p)$. Note for $0 < a < 1$, we have negative cooperativity, for $a = 1$, we have no cooperativity, and for $a > 1$, we have positive cooperativity.

Indeed, if ΔG_0 is the energy needed (or released) (i.e., the change in free energy) for binding the first F molecule to a G molecule, and if ΔG_1 is the energy used (or released) for binding an F molecule to the last unoccupied G molecule (whereupon $B = G_0 + B_0$), then we have the classical thermodynamic relations: $\Delta G_0 = -RT \log K(0)$, and $\Delta G_1 = -RT \log K(G_0 + B_0)$, where R is the gas constant (about 1.987 calories/degree/mole) and T is the absolute temperature. Thus, $K(G_0 + B_0)/K(0) = \exp(-(\Delta G_1 - \Delta G_0)/RT)$, and, by assumption, $K(G_0 + B_0)/K(0) = 1/(1 + p) = a$, so a has the interpretation: $a = K(B_0 + G_0)/K(0)$; it is the ratio of the equilibrium constant K with all G -sites occupied, to K with no occupied G -sites.

DeMeyts has observed that the relation $K(B_e) = K_0/(1 + ((1 - a)/a) \cdot B_e/(G_0 + B_0))$ may be used in the various equilibrium models given before to obtain the corresponding cooperative models. Thus, we substitute $K(B_e)$ for K to obtain models which now involve the parameters F_0 , G_0 , B_0 , a , and K_0 . Curve-fitting which yields a value for a obviously different from one, should be followed by fitting with a fixed to one. If the latter fit is clearly inferior, cooperative binding phenomena may be present.

In particular, for $a \neq 1$, the cooperative Michaelis-Menten(2) relation is:

$$B_e/(B_0 + G_0) = (-a(1 + K_0 F_e) + [a^2(1 + K_0 F_e)^2 + 4a(1 - a)K_0 F_e]^{1/2}) / (2(1 - a)).$$

The cooperative Scatchard equation is:

$$B_e/F_e = K_0(B_0 + G_0 - B_e) / (1 + (1 - a)B_e / (a(G_0 + B_0))).$$

The cooperative Hill equation is obtainable by substitution from the cooperative Michaelis-Menten (2) equation above, however, it can be plotted in MLAB, without using explicit algebra, as follows, assuming B_0 , G_0 , K_0 , and a are already set, with $a \neq 1$.

```
FCT BE(FE) = (B0+G0)*(-A*(1+K0*FE)+ \
    Sqrt(A*A*(1+K0*FE)^2+4*A*(1-A)*K0*FE))/(2*(1-A))
FUNCTION HILLT(BE) = LOG((BE/(G0+B0))/(1-BE/(G0+B0)))
LFEV = -12:2:.2
```

M = LFEV &' (HILLT ON BE ON EXP ON LFEV)
DRAW M

The Hill-plot matrix M has rows which are points of the form:

$$\log((B_e/(G_0 + B_0))/(1 - B_e/(G_0 + B_0))) \quad \text{vs.} \quad \log F_e.$$

Normally a Hill-plot, as defined above, is a straight line with slope 1, however, this is not the case for $a \neq 1$. DeMeyts has shown that, in general, the slope

$$d(\log((B_e/(G_0 + B_0))/(1 - B_e/(G_0 + B_0))))/d(\log F_e)$$

decreases when a decreases and increases when a increases, and attains its minimum value when $F_e = 1/K_0$, independently of a .