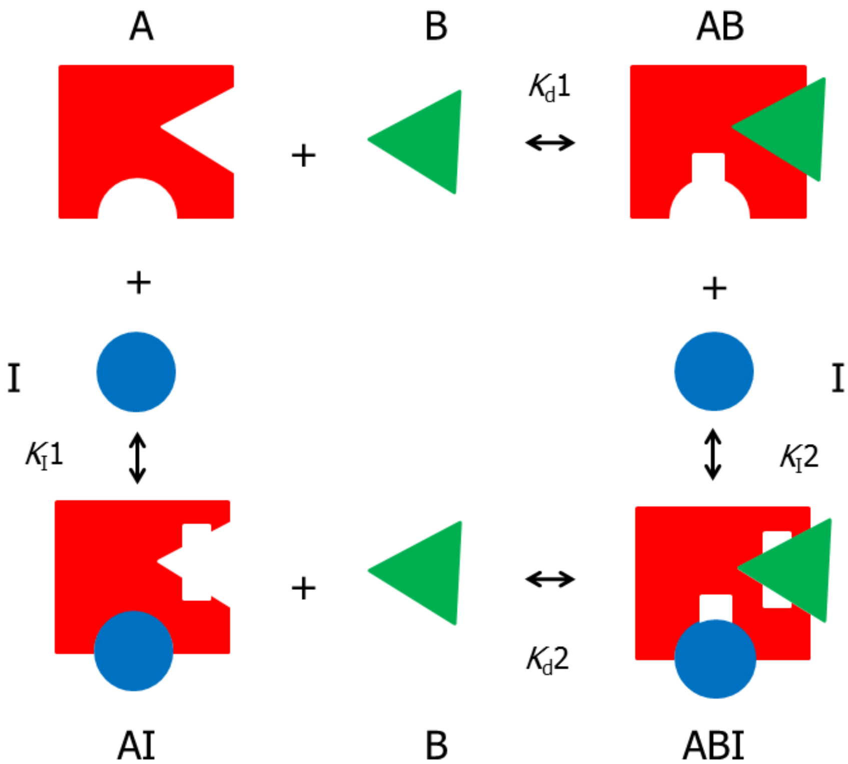
General inhibition model (Mixed competition)



Reactant B binding to reactant A is competitively and or non-competitively inhibited by inhibitor I

K_{d1} = Dissociation constant of A and B binding
K_{i1} = Dissociation constant of A and I binding
K_{d2} = Dissociation constant of AI and B binding
K_{i2} = Dissociation constant of AB and I binding

In the reaction sample you know…

Total reactant A concentration = A_0
Total reactant B concentration = B_0
Total inhibitor I concentration = I_0

However, you do not know…

Free reactant A concentration
Free reactant B concentration
Free inhibitor I concentration
Free product AB concentration
Free product ABI concentration
Free product AI concentration

Suppose you can indirectly determine concentration of product AB by a method which gives you values (activity, optical density, peak height, etc.) proportional to the concentration.

(Activity value) = k1 × [AB] (concentration).

If AI and ABI complex also contribute activity values (usually not!), actual activity values are

(Activity value) = $k1$ × [AB] (concentration) + $k2$ × [ABI] (concentration) + $k3$ × [AI] (concentration)

By performing curve fitting you try to obtain…

K_{d1}, K_{i1}, K_{d2}, K_{i2}, $k1$, $k2$, and $k3$ values

In this example (data sets) concentration of A_0 is fixed. Each data set was generated after measuring activity values in the presence of fixed concentration of B_0 after adding various concentration of I_0.

independent variable (x1 values) is I_0
dependent variable (y values) is activity.

There are two equations in each data set (total of four sets).

$$y = \frac{k1 \cdot B_0 \cdot A}{(K_{d1}+A)} + \frac{k3 \cdot I_0 \cdot A}{(K_{i1}+A)} + \left[k2 - \frac{k1 \cdot A}{(K_{d1}+A)} - \frac{k3 \cdot A}{(K_{i1}+A)}\right] \cdot [ABI]$$

$$A_0 - A - \frac{B_0 \cdot A}{(K_{d1}+A)} - \frac{I_0 \cdot A}{(K_{i1}+A)} + \left[\frac{A}{K_{d1}+A} + \frac{A}{K_{i1}+A} - 1\right] \cdot [ABI] = 0$$

(Newton Raphson equation)

$$[ABI] = \left(\frac{1}{2}\right) \cdot \left[B_0 + I_0 + k_{i2} \cdot \left(\frac{K_{d1}}{A} + 1\right) \cdot \left(\frac{A}{K_{i1}} + 1\right)\right] -$$

$$\left(\frac{1}{2}\right)\left[\sqrt{\left[B_0 + I_0 + k_{i2} \cdot \left(\frac{K_{d1}}{A} + 1\right) \cdot \left(\frac{A}{K_{i1}} + 1\right)\right]^2 - 4 \cdot A \cdot B_0}\right]$$

[ABI] = free ABI concentration

Press "**Fill Sheet with selected example data**" button to generate a new sheet filled with example data sets.

Described below are the input texts of equations according to “Curve Fitter Excel Add-in” syntax.

DataSet 1 (A_0 = 10, B_0 = 5)

f=10-n1-n1*5/(p1+n1)-n1*x1/(p2+n1)+(n1/(p1+n1)+n1/(p2+n1)-1)*(5+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((5+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*5))/2

y=p4*n1*5/(p1+n1)+p6*n1*x1/(p2+n1)+(p5-p4*n1/(p1+n1)-p6*n1/(p2+n1))*(5+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((5+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*5))/2

DataSet 2 (A_0 = 10, B_0 = 10)

f=10-n1-n1*10/(p1+n1)-n1*x1/(p2+n1)+(n1/(p1+n1)+n1/(p2+n1)-1)*(10+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((10+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*10))/2

y=p4*n1*10/(p1+n1)+p6*n1*x1/(p2+n1)+(p5-p4*n1/(p1+n1)-p6*n1/(p2+n1))*(10+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((10+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*10))/2

DataSet 3 (A_0 = 10, B_0 = 15)

f=10-n1-n1*15/(p1+n1)-n1*x1/(p2+n1)+(n1/(p1+n1)+n1/(p2+n1)-1)*(15+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((15+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*15))/2

y=p4*n1*15/(p1+n1)+p6*n1*x1/(p2+n1)+(p5-p4*n1/(p1+n1)-p6*n1/(p2+n1))*(15+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((15+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*15))/2

DataSet 4 (A_0 = 5, B_0 = 20)

f=10-n1-n1*20/(p1+n1)-n1*x1/(p2+n1)+(n1/(p1+n1)+n1/(p2+n1)-1)*(20+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((20+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*20))/2

y=p4*n1*20/(p1+n1)+p6*n1*x1/(p2+n1)+(p5-p4*n1/(p1+n1)-p6*n1/(p2+n1))*(20+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((20+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*20))/2

DataSet 5 (A_0 = 5, B_0 = 30)

f=10-n1-n1*30/(p1+n1)-n1*x1/(p2+n1)+(n1/(p1+n1)+n1/(p2+n1)-1)*(30+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((30+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*30))/2

y=p4*n1*30/(p1+n1)+p6*n1*x1/(p2+n1)+(p5-p4*n1/(p1+n1)-p6*n1/(p2+n1))*(30+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((30+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*30))/2

DataSet 6 (A_0 = 5, B_0 = 40)

f=10-n1-n1*40/(p1+n1)-n1*x1/(p2+n1)+(n1/(p1+n1)+n1/(p2+n1)-1)*(40+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((40+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*40))/2

y=p4*n1*40/(p1+n1)+p6*n1*x1/(p2+n1)+(p5-p4*n1/(p1+n1)-p6*n1/(p2+n1))*(40+x1+p3*(p1/n1+1)*(n1/p2+1)-sqrt((40+x1+p3*(p1/n1+1)*(n1/p2+1))^2-4*x1*40))/2

K_{d1} = p1
K_{i1} = p2
K_{i2} = p3
K_{d2} (dependent parameter, determined after curve fitting)
$k1$= p4
$k2$ = P5
$k3$ = P6
free reactant A concentration = n1 (determined by Newton Raphson method)

*In reality determine p1 and p4 values by fitting data set in the absence of inhibitor (I) separately. (Also determine p6 by an experiment in the absence of reactant B.) Then plug-in those value to the above equation and obtain p2, p3, and p5 values by curve fitting.

*This is a general treatment of inhibition model considering allosteric effect of reactant B and inhibitor I binding. In the case of competitive inhibition free ABI concentration is zero.