DERIVATION OF EQUATIONS FOR EQUILIBRIUM BINDING

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\text{-}S$$

Definitions

Dissociation constant

$K_d = [E][S]/[ES] = k_{-1}/k_1$

Association constant

$K_a = [ES]/[E][S] = k_1/k_{-1}$

Mass balance equations

$[E]_0 = [E] + [ES]$

$[S]_0 = [S] + [ES]$

K_d has units of concentration
k_{-1} is a first order rate constant with units of s^{-1}
k_1 is a second order rate constant with units of $M^{-1}s^{-1}$

Fraction of sites occupied:
fraction $= \theta = [ES]/[E]_0 = [ES]/([E]+[ES])$

$$\theta = \frac{[ES]}{[E]_0} = \frac{[ES]}{[E]+[ES]}$$

$$\theta = \frac{K_a[E][S]}{[E]+K_a[E][S]} = \frac{K_a[S]}{1+K_a[S]} = \frac{[S]}{1/K_a+[S]}$$

$$\theta = \frac{[S]}{K_d+[S]}$$

If [E] << Kd then one can assume that $[S] \cong [S]_0$ because S is not depleted due to binding to E.

$$\boxed{\theta = \frac{[S]_0}{K_d+[S]_0}}$$

☜ This is the equation for a hyperbola.
At $[S]_0 = K_d$ the fraction of sites bound is 0.5.

Linearized forms of the equation:

Double reciprocal plot:	Skatchard Plot
$1/\theta = \frac{[S]+K_d}{[S]} = 1 + \frac{K_d}{[S]}$ Or for multiple sites: $1/\upsilon = \frac{[S]+K_d}{[S]} = n + \frac{K_d}{[S]}$	$\theta = 1 - \frac{\theta K_d}{[S]}$ Or for multiple sites: $\theta = n - \frac{\upsilon K_d}{[S]}$ $\upsilon = \frac{\text{moles bound}}{\text{mole E}} = n\theta$

when you can not assume $[S]_0 \sim [S]$
but $[S]_0 = [S] + [ES]$

Derivation of quadratic equation--with no assumptions concerning substrate concentration.

$$fraction = \theta = [ES]/[E]_0 = [ES]/([E]+[ES])$$

☞ The derivation starts the same as above

$$\theta = \frac{K_a[E][S]}{[E]+K_a[E][S]} = \frac{K_a[S]}{1+K_a[S]} = \frac{[S]}{1/K_a+[S]}$$

☞ Fraction of sites bound relative to [S]

$$\theta = \frac{[S]}{K_d+[S]} = \frac{[S]_0-[ES]}{K_d+[S]_0-[ES]} = \frac{[ES]}{[E]_0}$$

☞ Substitution of $[S]=[S]_0-[ES]$

$$[ES](K_d+[S]_0)-[ES]^2 = [E]_0[S]_0-[ES][E]_0$$

$$[ES]^2-[ES](K_d+[S]_0+[E]_0)+[E]_0[S]_0$$

☞Form of equation require solution as the roots of the quadratic equation

Solution is quadratic equation:

$$ax^2+bx+c=0$$

solution provided by the roots of the quadratic

$$x = \frac{-b \pm \sqrt{b^2-4ac}}{2a}$$

$$K_d = \frac{[E][S]}{[ES]}$$

$$[ES] = \frac{(E_0+S_0+K_d)-\sqrt{(E_0+S_0+K_d)^2-4\cdot E_0\cdot S_0}}{2}$$

*

$$\boxed{\theta = \frac{(E_0+S_0+K_d)-\sqrt{(E_0+S_0+K_d)^2-4\cdot E_0\cdot S_0}}{2\cdot E_0}}$$

This equation must be used rather than the hyperbola whenever the enzyme concentration is comparable or greater than the dissociation constant. As a general rule, if $[E]_0$ is less than 5 times the K_d, the hyperbolic fit is probably adequate.

Fluorescence data

$$F = F_0 + \Delta F \cdot \theta$$

where $\Delta F = F_\infty - F_0$

and θ is defined by either:

☞ This equation normalizes the observable signal to a scale that can be related to fractional occupancy, θ.

$$\theta = \frac{[S]_0}{K_d+[S]_0}$$

OR…..

$$\theta = \frac{(E_0+S_0+K_d)-\sqrt{(E_0+S_0+K_d)^2-4\cdot E_0\cdot S_0}}{2\cdot E_0}$$

The same equations could be applied to any signal, such as counts per minute or other measure of bound substrate.

Multiple Binding Sites:

Reaction step	*Association constant*
$E + S \underset{}{\overset{K_1}{\rightleftarrows}} ES$	$[ES] = K_1[E][S]$
$ES + S \underset{}{\overset{K_2}{\rightleftarrows}} ES_2$	$[ES_2] = K_2[ES][S] = K_1K_2[E][S]^2$
$ES_2 + S \underset{}{\overset{K_3}{\rightleftarrows}} ES_3$	$[ES_3] = K_3[ES_2][S] = K_1K_2K_3[E][S]^3$
$ES_3 + S \underset{}{\overset{K_4}{\rightleftarrows}} ES_4$	$[ES_4] = K_4[ES_3][S] = K_1K_2K_3K_4[E][S]^4$

Solution of fraction of sites occupied for a two-step binding sequence.

Mass balance equations:

$$[\mathrm{E}]_0 = [E] + [ES] + [ES_2]$$

$$[S]_0 \simeq [S] \text{ (negligible amount bound)}$$

Fraction of sites bound:

$$\theta = ([ES_1] + [ES_2])/[E]_0$$

$$= \frac{[ES_1] + [ES_2]}{[E] + [ES_1] + [ES_2]}$$

Substitution of bound states:

$$[ES_1] = K_1[E][S]$$

$$[ES_2] = K_2[ES_1][S] = K_1K_2[E][S]^2$$

$$\theta = \frac{K_1[E][S] + K_1K_2[E][S]^2}{[E] + K_1[E][S] + K_1K_2[E][S]^2}$$

$$\boxed{\theta = \frac{K_1[S] + K_1K_2[S]^2}{1 + K_1[S] + K_1K_2[S]^2}}$$

Fraction of sites bound

If the equations are defined for moles of substrate bound per mole of dimers instead of per mole of active sites, the equations becomes:

$$\upsilon = \frac{K_1[S] + 2K_1K_2[S]^2}{1 + K_1[S] + K_1K_2[S]^2} \quad \text{where } \upsilon = \frac{\text{moles S bound}}{\text{mole of dimers}}$$

Accordingly, the binding equation ranges from 0-2 moles bound rather than from 0-1 fraction of sites occupied.