Fitting Equations in Analysis

Protein-Ligand Fast Exchange Kd Equation

This equation is for fitting Kd using shifts measured at varying concentrations of ligand (and sometimes also protein).

We have the reaction $A + B \rightleftharpoons AB$. We take A to be the protein (or other molecule that binds) and B to be the ligand. We vary the amount of B and normally we keep the amount of A fixed.

Let $[A]$ be the concentration of A in the unbound state, $[B]$ be the concentration of B in the unbound state and $[AB]$ be the concentration of the protein-ligand complex.

Let $a = [A] + [AB]$ be the total concentration of A. Let $b = [B] + [AB]$ be the total concentration of B.

The equilibrium dissociation constant is

$$K_d = \frac{[A][B]}{[AB]}$$

We have

$$\begin{aligned}
[A] &= \frac{K_d[AB]}{[B]} \\
&= \frac{K_d(a - [A])}{[B]} \\
&= \frac{K_d(a - [A])}{b - [AB]} \\
&= \frac{K_d(a - [A])}{b - a + [A]}
\end{aligned}$$

Multiply up to get

$$[A](b - a + [A]) = K_d(a - [A])$$

and so

$$[A]^2 + (b - a + K_d)[A] - aK_d = 0$$

Or in other words

$$
\begin{aligned}
[A] &= \frac{-(b-a+K_d)+\sqrt{(b-a+K_d)^2+4aK_d}}{2} \\
&= \frac{-(b-a+K_d)+\sqrt{(b+a+K_d)^2-4ab}}{2}
\end{aligned}
$$

(We cannot have the other root because it would give a negative $[A]$, as can be seen from the first line above.) Then

$$
\frac{a-[A]}{a} = \frac{b+a+K_d-\sqrt{(b+a+K_d)^2-4ab}}{2a}
$$

The percentage of bound A is

$$
r_{bound} = \frac{[AB]}{a} = \frac{a-[A]}{a}
$$

and the percentage of unbound A is

$$
r_{free} = 1 - r_{bound}
$$

Let δ_{bound} be the chemical shift of the bound A and δ_{free} be the chemical shift of the unbound A.

For fast exchange we have that the observed chemical shift is

$$
\begin{aligned}
\delta_{obs} &= r_{bound}\delta_{bound} + r_{free}\delta_{free} \\
&= r_{bound}\delta_{bound} + (1-r_{bound})\delta_{free} \\
&= \delta_{free} + (\delta_{bound}-\delta_{free})r_{bound} \\
&= \delta_{free} + (\delta_{bound}-\delta_{free})\left(\frac{a-[A]}{a}\right) \\
&= \delta_{free} + (\delta_{bound}-\delta_{free})\left(\frac{b+a+K_d-\sqrt{(b+a+K_d)^2-4ab}}{2a}\right)
\end{aligned}
$$

We normally know what δ_{free} is and so we introduce

$$
\Delta\delta_{obs} = \delta_{obs} - \delta_{free}
$$

and similarly

$$
\Delta\delta_{\infty} = \delta_{bound} - \delta_{free}
$$

in which case the equation becomes

$$\Delta\delta_{obs} = \Delta\delta_{\infty}\left(\frac{b + a + K_d - \sqrt{(b + a + K_d)^2 - 4ab}}{2a}\right)$$

We know a and b. Sometimes we know δ_{bound} in which case the only thing left to fit is K_d. Sometimes we do not know δ_{bound} in which case we have to fit that as well (so equivalently $\Delta\delta_{\infty}$).

Let

$$\begin{aligned} A &= \Delta\delta_{\infty}/2 \\ B &= 1 + K_d/a \\ x &= b/a \\ y &= \Delta\delta_{obs} \end{aligned}$$

Then the above equation becomes

$$y = A\left(B + x - \sqrt{(B + x)^2 - 4x}\right)$$

which is the equation used in Analysis. We have shift measurements at various b. We fit both A and B and we also use a weighted sum over the (normally two) dimensions of the shift to make a single scalar. This is not optimal. The ratio y/A is independent of dimension. In other words, all dimensions should (or could) provide an estimate of K_d, and combining the shifts together loses information.

Note that

$$K_d = a(B - 1)$$

This fitting only makes sense if b is kept constant throughout the experiments. If b also varies then we have that y has two dependent variables (in effect, a and b). The Analysis code would need some changing to cope with that.

A description of the fitting equation is on the web at

$$http://structbio.vanderbilt.edu/chazin/wisdom/kdcalc.htm$$

The correspondence with the formulae in that webpage is:

$$
\begin{aligned}
Q_a &= \delta_{obs} \\
Q_0 &= \delta_{free} \\
Q_{max} &= \delta_{bound} \\
[P_{tot}] &= a \\
[A_{tot}] &= b
\end{aligned}
$$

Monomer-Dimer Fast Exchange Kd Equation

We have the reaction $A + A \rightleftharpoons AA$. Let Let $[A]$ be the concentration of the monomer and $[AA]$ the concentration of the dimer. Let $a = [A] + 2[AA]$ be the total concentration of A.

The equilibrium dissociation constant is

$$K_d = \frac{[A]^2}{[AA]}$$

Define the ratio

$$r = \frac{[AA]}{a}$$

or in other words

$$[AA] = ra$$

Then we also have

$$[A] = a - 2[AA] = (1 - 2r)a$$

(Note that $0 \leq r \leq 1/2$.) Therefore we have

$$K_d = \frac{(1 - 2r)^2}{r} a$$

or in other words

$$4r^2 - 4r + 1 = (1 - 2r)^2 = \frac{K_d}{a} r$$

and so

$$r^2 - \left(1 + \frac{K_d}{4a}\right) r + \frac{1}{4} = 0$$

And thus

$$\begin{aligned} r &= \frac{1}{2}\left(1 + \frac{K_d}{4a} - \sqrt{\left(1 + \frac{K_d}{4a}\right)^2 - 1}\right) \\ &= \frac{1}{8a}\left(K_d + 4a - \sqrt{(K_d + 4a)^2 - 16a^2}\right) \end{aligned}$$

The positive root cannot be taken because that would make $r > 1/2$.

Let δ_A be the chemical shift of the monomer and δ_{AA} be the chemical shift of the dimer, and define $\Delta\delta_\infty = \delta_{AA} - \delta_A$. In general we do not know either δ_A or δ_{AA}.

For fast exchange we have that the observed chemical shift is

$$\begin{aligned} \delta_{obs} &= \frac{[A]\delta_A + 2[AA]\delta_{AA}}{a} \\ &= (1 - 2r)\delta_A + 2r\delta_{AA} \\ &= \delta_A + 2r(\delta_{AA} - \delta_A) \\ &= \delta_A + 2r\Delta\delta_\infty \\ &= \delta_A + \frac{\Delta\delta_\infty}{4a}\left(K_d + 4a - \sqrt{(K_d + 4a)^2 - 16a^2}\right) \end{aligned}$$

There is no point subtracting anything here since we do not know either δ_A or δ_{AA} in general.

Let

$$\begin{aligned} A &= \Delta\delta_\infty \\ B &= K_d \\ C &= \delta_A \\ x &= a \\ y &= \delta_{obs} \end{aligned}$$

Then the above equation becomes

$$\begin{aligned} y &= A\left(B + 4x - \sqrt{(B + 4x)^2 - 16x^2}\right)/4x + C \\ &= A\left(1 + B/4x - \sqrt{(1 + B/4x)^2 - 1}\right) + C \end{aligned}$$

which is the equation used in Analysis.