

CH 347

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Fitting of Binding Data using Nonlinear Least Squares

Fitting of data is always possible if you can simulate the data with a mathematical function or routine and a given set of parameters. The simulated and observed data are compared, and the fitting parameters are adjusted to minimize the differences between the two (i.e. the residuals).

Simulation and fitting of binding data obtained directly (e.g. by equilibrium dialysis where you know the free ligand concentration and E.I concentration) is straightforward since you can calculate the binding density directly from the experimental data. This is then fit by the binding isotherm equation as discussed in class and in the binding handout.

The fitting of indirect binding data, e.g. that obtained by observing a change in fluorescence of a protein as it is titrated with ligand, requires a different function. The fraction of protein containing bound ligand is assumed to be directly proportional to the change in fluorescence. We know only the total amount of protein that we start with, the total amount of ligand added at each point in the titration, and the magnitude of the observed signal (e.g. fluorescence). We can use this data to obtain the binding constant (or dissociation constant) as well as the maximal change in fluorescence.

The free ligand concentration is given by

$$I = I_{\text{total}} - E.I \quad 1.$$

and total E is given by

$$E_{\text{total}} = E.I + E \quad 2.$$

(Square brackets indicating concentrations are eliminated for clarity. For example, I should be read as [I]).

The binding constant is defined as

$$K = \frac{E.I}{E * I} \quad 3.$$

from which we can write

$$E = \frac{E.I}{K * I} \quad 4.$$

Substitution of equation 1 into 3, and equation 3 into 2 yields

$$E_{\text{total}} = E.I + \frac{E.I}{K(I_{\text{total}} - E.I)}$$

Solving for E.I gives us

$$EI = \frac{1 + K * E_{total} + K * I_{total} - \sqrt{-4K^2 E_{total} I_{total} + (1 + KE_{total} + KI_{total})^2}}{2K}$$

This equation gives us the concentration of E.I at any point in the titration in terms of total concentrations of E and I as well as the binding constant. We have removed the need to know the free concentration of I. The binding density $\nu = E.I/E_{total}$.

The observed fluorescence is then given by $F_{max} * \nu$.

These equations allow us to simulate the observed fluorescence (or any signal proportional to the binding) as a function of the total amount of E and I added if we assume a value for the binding constant. Or we can fit binding data with this equation by adjusting K to minimize the residuals in a plot of binding density (or spectroscopic signal) as a function of total ligand added to a known amount of protein.