

### Derivation of General Function for Single-Site Binding

(based on Alan Cooper, Biophysical Chemistry, pp. 109-110)

We are aiming to derive an equation for the concentration of protein-ligand complex in terms of the total concentrations of ligand and protein, so that we can predict the degree of complex formation in solution from the concentrations of added ligand and protein.

From the equilibrium constant definition:

$$K_D = \frac{[P][L]}{[PL]} \Rightarrow [P] = \frac{K_D \cdot [PL]}{[L]} \quad (1)$$

Then derive the mass-balance equations for the total concentrations of ligand ( $C_L$ ) and protein ( $C_P$ ) in terms of the concentrations of free ligand ( $L$ ), free protein ( $P$ ), and protein-ligand complex ( $PL$ ):

$$C_L = [L] + [PL] \Rightarrow [L] = C_L - [PL] \quad (2)$$

$$C_P = [P] + [PL] \quad (3)$$

Substituting (1) for  $[P]$  in (3) gives:

$$C_P = \frac{K_D [PL]}{[L]} + [PL] \quad (4)$$

Substituting (2) for  $[L]$  in (4) gives:

$$C_P = \frac{K_D [PL]}{C_L - [PL]} + [PL] \quad (5)$$

Rearranging that equation to get a quadratic for  $[PL]$  requires a few steps...

Multiply (5) through by  $C_L - [PL]$ :

$$C_P C_L - C_P [PL] = K_D [PL] + C_L [PL] - [PL]^2 \quad (6)$$

Rearrange:

$$C_P C_L - C_P [PL] - K_D [PL] - C_L [PL] = -[PL]^2 \quad (7)$$

Factorise the terms in  $[PL]$  and rearrange:

$$[PL]^2 - (C_P + C_L + K_D)[PL] + C_P C_L = 0 \quad (8)$$

This is a quadratic equation with  $a = 1$ ,  $b = -(C_P + C_L + K_D)$  and  $c = C_P C_L$ .

The physically correct solution from the quadratic formula is:

$$[PL] = \frac{(C_P + C_L + K_D) - \sqrt{(C_P + C_L + K_D)^2 - 4C_P C_L}}{2} \quad (9)$$

This needs modification for fitting *actual data*. Here we discuss the case for a labelled ligand that changes signal upon binding. The fraction of the total ligand concentration ( $C_L$ ) that is in the  $PL$  complex can be called the fraction bound ( $F_b$ ):

$$F_b = \frac{[PL]}{C_L} \quad (10)$$

Combining (9) and (10) we get:

$$F_b = \frac{(C_P + C_L + K_D) - \sqrt{(C_P + C_L + K_D)^2 - 4C_P C_L}}{2C_L} \quad (11)$$

We don't actually directly measure the fraction bound, but rather monitor the observed signal from the ligand ( $S_{obs}$ ). This signal is the average of the signal from the free ligand ( $S_f$ ) and that of the bound ligand ( $S_b$ ), weighted by the populations of the free and bound state (calculated from  $F_b$ ):

$$S_{obs} = S_b \cdot F_b + S_f \cdot (1 - F_b) \quad (12)$$

Rearranged another way,  $F_b$  is used to scale the difference between the free and bound signals:

$$S_{obs} = S_f + (S_b - S_f) \cdot F_b \quad (13)$$

Substituting (11) for  $F_b$  in (13), the total fitting function is therefore:

$$S_{obs} = S_f + (S_b - S_f) \cdot \left( \frac{(C_P + C_L + K_D) - \sqrt{(C_P + C_L + K_D)^2 - 4C_P C_L}}{2C_L} \right)$$