\[ P + A \Rightarrow PA \]

Assuming \( Q_{eq}^0 = C_i^0 \)

Mass Balance

\[
P_T = [P] + [PA] \quad \text{A}_T = [A] + [PA]
\]

\[ K = \frac{[PA]}{[P][A]} \]

Three unknowns: \([P], [A], [PA]\)

Three equations

Solve exactly: let \( x = [PA] \)

\[
[P] = P_T - x \quad [A] = A_T - x
\]

\[ K[P][A] = [PA] \Rightarrow K(P_T - x)(A_T - x) = x
\]

\[ x^2 - (P_T + A_T + \frac{1}{K})x + P_T A_T = 0
\]

\[ x = \frac{(P_T + A_T + \frac{1}{K}) \pm \sqrt{(P_T + A_T + \frac{1}{K})^2 - 4P_T A_T}}{2}
\]

More commonly, assume \( A_T \gg P_T \) (ligand in large excess)

Thus:

\[
[P] \approx P_T
\]

\[ [A] = A_T - x
\]

\[ K P_T (A_T - x) = x
\]

\[ K P_T A_T - K P_T x - x = 0
\]

\[ x = \frac{K P_T A_T}{1 + K P_T} = \frac{P_T A_T}{\frac{1}{K} + P_T}
\]
\[ K = \frac{A_{\text{bound}}}{(P_T - A_{\text{bound}} \times A_{\text{free}})} \]

If we define:
\[ \lambda = \frac{A_{\text{bound}}}{P_T} \]

\[ K = \frac{A_{\text{bound}}}{(1 - \frac{A_{\text{bound}}}{P_T} \times A_{\text{free}})} \]

\[ \lambda = 0 \rightarrow 1 \]

fractional occupancy

Re-arrange:

\[ \frac{\lambda}{[A]} = K(1 - \lambda) \]

This was written for a protein, P, with only one binding site for A.

If there N identical and independent binding sites, then

\[ \frac{\lambda}{A} = K(1 - \frac{\lambda}{N}) \]

\[ \frac{\lambda}{A} = K(N - \lambda) \]

Scatchard Equation

\( \lambda \) = number of bound A per P
\( N \) = number of sites per P (the maximum of \( \lambda \) possible)

\( \frac{\lambda}{N} \) = Fraction of total sites occupied
Cooperative (and Anticooperative) Binding

Cooperative Binding $\Rightarrow$ Binding of first ligand makes binding of subsequent ligands stronger (lower $K_A$)

Special case $\Rightarrow$ "All or None"
Binding of first makes binding of subsequent infinitely strong. Intermediate populations don't exist.

Examples

Hill Plot

From before $\Rightarrow \frac{Y}{1-Y} = K_A \frac{f}{1-f}$

For interactive sites:

$\frac{f}{1-f} = K_A^n$  \hspace{1cm} (Hill coefficient (not constant across titration - indicates cooperativity)

$\log \frac{f}{1-f} = n \log A + \log K$

Good for non-interaction sites
Back to Equilibrium Dialysis

1) Radiolabel $A$
2) Dialyze to equilibrium
3) Separate contents of side 1 and side 2
   Using radioactivity of $^*A$ determine total $^*A$
on each side.

\[
\begin{align*}
\text{In compartment } \frac{(1)}{2} & \Rightarrow A + PA \\
\text{In compartment } (2) & \Rightarrow A \\
\text{Measure } [\text{PROTEIN}] \text{ in side } (1) & \Rightarrow P + PA = P_{\text{tot}} \\
& \text{ (or know it from setup)}
\end{align*}
\]

Charged solutes $\Rightarrow A^+$
and proteins

Originally binding strength only
determined distribution of
ligand on each side.
Now we have an additional
requirement of charge neutrality.