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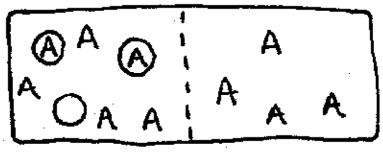


$$P + A \rightleftharpoons PA$$

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

Assuming $a_i^o = C_i^o$ $\longrightarrow$

Three unknowns: $[P]$, $[A]$, $[PA]$

Mass Balance

$$P_T = [P] + [PA]$$
$$A_T = [A] + [PA]$$

Three equations

Solve exactly: let $x = [PA]$

$$[P] = P_T - x \qquad [A] = A_T - x$$

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

$$x^2 - (P_T + A_T + 1/K)x + P_T A_T = 0$$

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

More ~~generally~~ Commonly, assume $A_T \gg P_T$
then

Ligand in large excess

$$[P] \approx P_T$$
$$[A] = A_T - x$$

$$K P_T (A_T - x) = x$$
$$K P_T A_T - K P_T x - x = 0$$

$$\longrightarrow \quad x = \frac{K P_T A_T}{1 + K P_T} = \frac{P_T A_T}{1/K + P_T}$$

$$\mathcal{K} = \frac{\overbrace{A_{BOUND}}^{=PA}}{\underbrace{(P_T - A_{BOUND})}_{P_{free}}(A_{free})}$$ * Exact again

If we define:

$$\nu = \frac{A_{BOUND}}{P_T} \qquad \left(= \frac{C_A(\text{bound})}{C_M} \right)$$ ← IN BOOK

fraction of total P's that have A bound

$\nu = 0 \rightarrow 1$ fractional occupancy

$$\mathcal{K} = \frac{A_{BOUND}/P_T}{\left(1 - \frac{A_{BOUND}}{P_T}\right)(A_{free})}$$

$$\mathcal{K} = \frac{\nu}{(1-\nu)A_{free}} \qquad A_{free} = [A]$$

Re-arrange

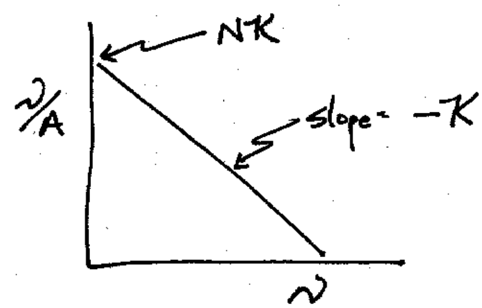
$$\nu/[A] = \mathcal{K}(1-\nu)$$

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

If there N identical and independent binding sites, THEN

$$\frac{\nu/N}{A} = \mathcal{K}\left(1 - \frac{\nu}{N}\right)$$

$$\nu/A = \mathcal{K}(N-\nu) \leftarrow \text{Scatchard Equation}$$



ν = number of bound A per P

N = number of sites per P (the maximum of ν possible)

ν/N = Fraction of total sites occupied

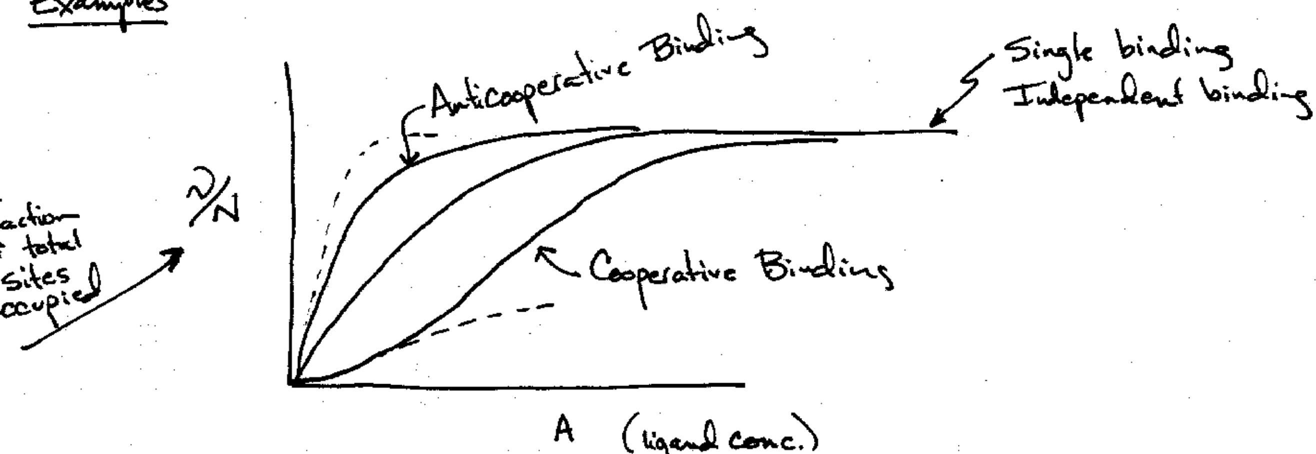
Cooperative (and Anticooperative) Binding

NOT independent

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

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

Examples



SKIP

HILL PLOT

From before $\Rightarrow$ $\dfrac{\nu/N}{1-\nu/N} = KA = \dfrac{f}{1-f}$

GOOD FOR NON-INTERACTING SITES

For interactive sites:

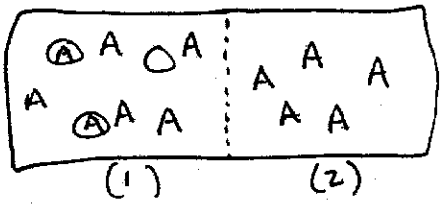
$$\frac{f}{1-f} = K A^{n}$$

n ← Hill coefficient (not constant across titration - indicates cooperativity).

K ↑ a constant

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

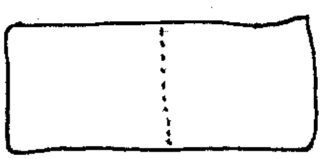
Back to Equilibrium Dialysis



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.

In compartment (1) $\Rightarrow$ A + PA
In compartment (2) $\rightarrow$ A
Measure [PROTEIN] in side (1) $\rightarrow$ $P + PA = P_{TOT}$
(or know it from setup)

Charged solutes and proteins $\Rightarrow$ A^+



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