Simple Binding

$P + L \rightleftharpoons PL$ reaction equilibrium

P: protein L: ligand (ligand can be another protein)

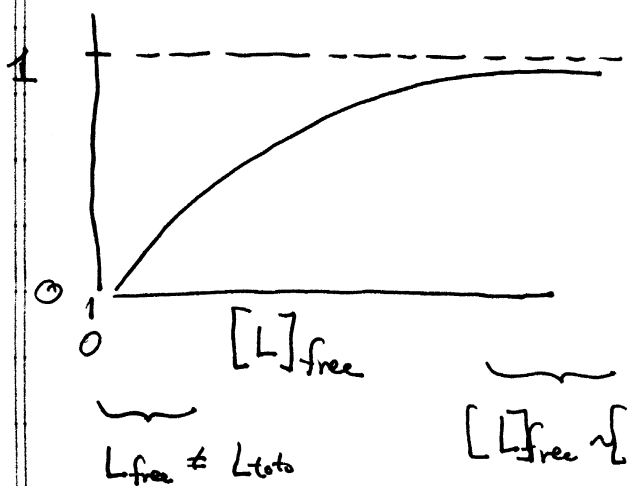
$K = \dfrac{[PL]_{eq}}{[P]_{eq}[L]_{eq}}$ (M^{-1}) $\qquad \Delta G^\circ = -RT \ln K$

$\Delta G = \Delta G^\circ + RT \ln Q \qquad Q = \dfrac{[PL]}{[P][L]}$ not at equilibrium

$\Delta G = \Delta G^\circ$ $\qquad Q = 1$ at standard state

$\bar{\upsilon} = \dfrac{[PL]}{[P] + [PL]}$ average number of ligand molecules bound

$[L] = 0 \quad \bar{\upsilon} = 0 \qquad [L] \to \infty$ then $\bar{\upsilon} = 1$



$$\bar{\nu} = \frac{K[P][L]}{[P] + K[P][L]} = \frac{K[L]}{1 + K[L]}$$

helpful, but not necessary information: ~~total~~ protein concentration, # of binding sites on protein, whether sites are equivalent or not (equal in affinity), protein molecular weight

binding experiment can help figure some of this stuff out.

Multiple Binding Sites on Protein

Myoglobin: 1 O_2 binding site
Hemoglobin: 4 O_2 binding sites
Antibodies: 2 antigen binding sites
Avidin/Streptavidin: 4 biotin binding sites

suppose there are n sites

The overall reaction is $P + nL \rightleftharpoons PL_n$

Overall binding constants, call them 'β'

$$\beta_1 = \frac{[PL]}{[P][L]} \qquad P + L \rightleftharpoons PL$$

$$\beta_2 = \frac{[PL_2]}{[P][L]^2} \qquad P + 2L \rightleftharpoons PL_2$$

$$\beta_3 = \frac{[PL_3]}{[P][L]^3} \qquad P + 3L \rightleftharpoons PL_3$$

$$\vdots$$

$$\beta_n = \frac{[PL_n]}{[P][L]^n} \qquad P + nL \rightleftharpoons PL_4$$

overall equation for 'n' binding sites

Stepwise, K_i = association constant (macroscopic)

$$K_1 = \frac{[PL]}{[P][L]} \qquad P + L \rightleftharpoons PL$$

$$K_2 = \frac{[PL_2]}{[PL][L]} \qquad PL + L \rightleftharpoons PL_2$$

$$K_3 = \frac{[PL_3]}{[PL_2][L]} \qquad PL_2 + L \rightleftharpoons PL_3$$

$$\vdots$$

$$K_n = \frac{[PL_n]}{[PL_{n-1}][L]} \qquad PL_{n-1} + L \rightleftharpoons PL_n$$

General Equation for average bound ($\bar{\upsilon}$) vs. free Ligand

$$\bar{\upsilon} = \frac{\sum_{i=1}^{n} i\beta_i [P][L]^i}{\sum_{i=1}^{n} \beta_i [P][L]^i} \qquad \frac{\text{(all bound ligand)}}{\text{(total protein)}}$$

$$\bar{\upsilon} = \frac{\sum_{i=1}^{n} i\beta_i [L]^i}{\sum_{i=0} \beta_i [L]^i}$$

Adair Equation

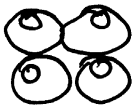
Adair Equation - no assumption about independence, equivalency, cooperativity

(I) Assome All site equivalent & independent

independent: no interaction between sites (non-cooperative)

However K_i are <u>not</u> equal

The <u>macroscopic</u> stepwise equilibrium does not account for the different ways of creating microscopic states



one empty state

four micro states with 1 Ligand

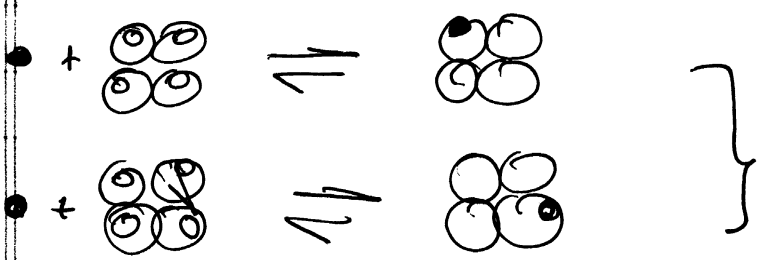
in general the number of ~~ways~~ micro states with i ligands bound to n sites total.

$$N_{i,n} = \frac{n!}{(n-i)!\,i!}$$

n = number of sites
i = sites filled
$n-i$ = vacancies

call the microscopic constant k

microstate 1



microstate 2

k's are equal

etc.

$$[PL_i]_e = k^i [P][L]^i$$

$$[PL_i] = \frac{n!}{(n-i)!\,i!}\,[P]\,k^i [L]^i$$

$$\bar{v} = \frac{\sum_{i=1}^{n} i \,\frac{n_i!}{(n-i)!\,i!}\,(k[L])^i}{\sum_{i=0}^{n} \frac{n!}{(n-i)!\,i!}\,(k[L])^i}$$

where $\beta_i = \frac{n_i}{(n-i)!\,i!}\,k_i$

denominator is the binomial expression

$$\sum_{i=0}^{n} \frac{n!}{(n-i)!\,i!}\,(k_i[L])^i = (1 + k[L])^n$$

$N_{i,n}$ is the binomial coefficient

1 2 1

1 3 3 1

1 4 6 4 1

etc.

numerator

$$(1 + k[L])^{n-1} = \sum_{i=0}^{n-1} \frac{(n-1)!}{(n-1-i)!\,i!} (k[L])^i$$

$$= \frac{1}{nk[L]} \sum_{i=0}^{n-1} \frac{n!\,(i+1)}{(n-(i+1))!\,(i+1)!} k^{i+1} [A]^{i+1}$$

substitute $j = i + 1$

$$= \frac{1}{nk[L]} \sum_{j=1}^{n} \frac{n!\,j}{(n-j)!\,j!} k^j L^j$$

$$\frac{1}{nk[L]} = \sum_{i=1}^{n} i \frac{n!}{(n-i)!\,i!} (k[L])^i$$

$$\bar{v} = \frac{nk[L](1 + k[L])^{n-1}}{(1+k[L])^n}$$

$$\boxed{\bar{v} = \frac{nk[L]}{1 + k[L]}}$$

$$\boxed{\vartheta = \frac{\bar{v}}{n} = \frac{k[L]}{1 + k[L]}}$$

ϑ, fraction of sites occupied

(i) $\bar{\upsilon} = \sum_{s=1}^{N} \bar{\upsilon}_s$

N = all ~~sites~~ sets of sites

(ii) $\bar{\upsilon}_s = \dfrac{n_s k_s [L]}{1 + k_s [L]}$

(iii) $\bar{\upsilon} = \sum_{s=1}^{N} \dfrac{n_s k_s [L]}{1 + k_s [L]}$

k_s intrinsic constant of each set

n_s number of sites of type s

(iv) $\bar{\upsilon} = \dfrac{n_1 k_1 [L]}{1 + k_1 [L]} + \dfrac{n_2 k_2 [L]}{1 + k_2 [L]}$

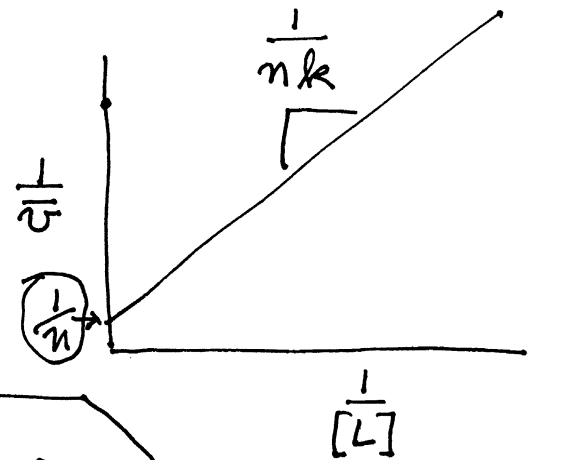
Suppose there are multiple sets of sites, then $\bar{\upsilon}$ is the sum of the different sets of sites. Above (in (i)) the average extent of binding is the sum of average binding over all sets. (N sets, $\bar{\upsilon}_s$ average binding in each set.) Each set, s, obeys single-set-of-sites model (ii). The average degree of binding is the sum over all these sets (iii). For the specific case of two sets, there are two values of each parameter, number of sites, n_1 and n_2, and binding constants, k_1 and k_2

Representations of Linearized Data

(1) Double reciprocal plot

$$\frac{1}{v} = \frac{1 + k[L]}{nk[L]}$$

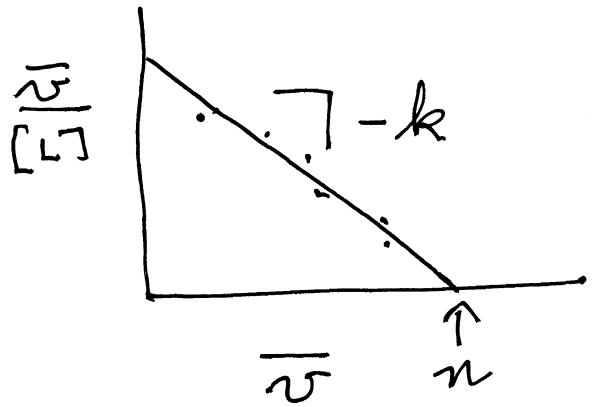
$$\frac{1}{v} = \frac{1}{n} + \frac{1}{nk[L]}$$



$$v\,(1 + k[L]) = nk[L]$$

$$\frac{v}{[L]}\,(1 + k[L]) = nk$$

(2) $\frac{v}{[L]} = nk - vk$ Scatchard Plot



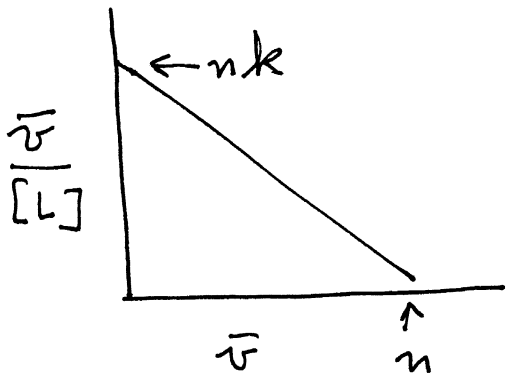
Straight line

means: independent & equivalent set of sites

"Single Set of Sites"

A linear Scatchard Plot has a single microscopic binding constant: the slope is $-k$.

$$\frac{\bar{\upsilon}}{[L]} = nk - \bar{\upsilon}k$$



The slope is $-k$, the x-intercept is the number of sites, n, at saturating concentrations of ligand $[L] \Rightarrow$ large, $\bar{\upsilon}/[L] \Rightarrow 0$, $\bar{\upsilon} \Rightarrow n$.

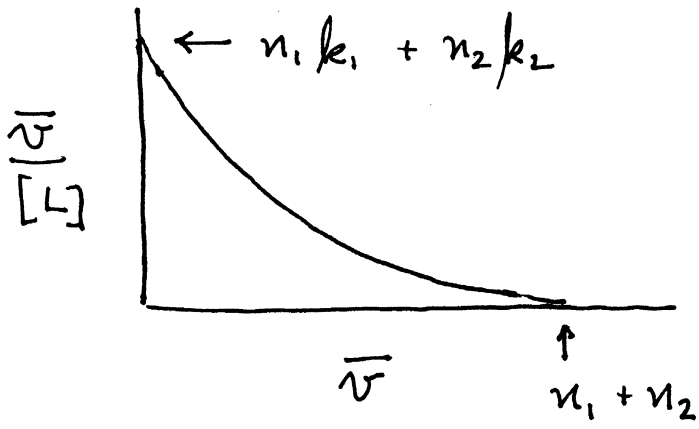
A linearized representation of the ~~plot~~ data, provides an immediate indication of any deviation from a single-set-of-sites binding (one microscopic constant).

Consider two sets of sites $\bar{\upsilon} = \dfrac{n_1 k_1 [L]}{1 + k_1[L]} + \dfrac{n_2 k_2 [L]}{1 + k_2[L]}$

with $k_1 \neq k_2$.

In this case one set of sites, the high affinity sites will fill first and then the low affinity sites. The slope will be steeper when $\bar{\upsilon}$ is small and lessen as $\bar{\upsilon}$ approaches saturation, $\bar{\upsilon} = n_1 + n_2$ at saturation.

Expectation: Multiple sets of sites will produce a curved Scatchard plot:

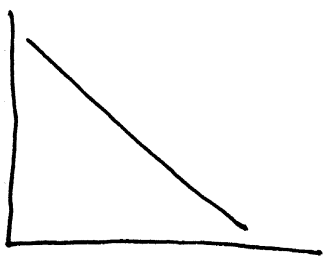


(1) The 'concave upward' Scatchard plot is characteristic of multiple sets of sites.

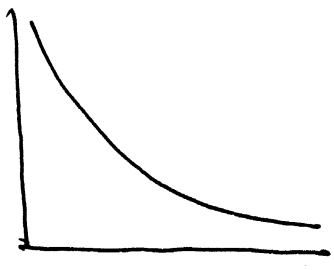
Other types of binding situations can lead to curvature in a Scatchard plot. Some common examples are, (for two site binding, for simplicity)

(2) negative cooperative interactions: 'concave upward'

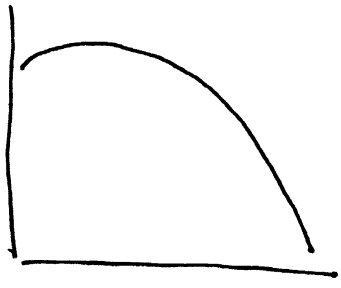
(3) positive cooperative interactions between sites, when the intrinsic affinities of sites 1 and 2 are ~~equal~~ equal: 'concave downward'



(1) single set of sites



(2) multiple sets of sites
(4) positive coop unequal intrinsic affinity



(3) positive coop. equal intrinsic affinity

(4) The fourth situation can give a concave upward curve, ~~desp~~ and have positive cooperativity

The intrinsic affinity at the first site k_1, increases affinity at the second site, but the increase is insufficient to make k_2 larger than k_1.

Without ligand bound $k_1 \gg k_2$
With site one filled $k_1 > k_2'$ $\quad k_2' > k_2$

Models of Cooperativity

(1) Consider the simple case, in which two ligands bind

$$P + 2L \rightleftharpoons PL_2$$

Only two forms of protein are present. P without ligand
P with two ligands bound PL_2

$$\bar{v} = \frac{2k[L]^2}{1 + k[L]^2}$$

This is the 'ALL or NONE' model
- not terribly realistic.

2 Hill Equation

$$\vartheta = \frac{\bar{\upsilon}}{n_s} = \frac{k[L]^{n_H}}{1 + k[L]^{n_H}} \quad ; \quad \bar{\upsilon} = \frac{n_s k[L]^{n_H}}{1 + k[L]^{n_H}}$$

ϑ = fractional saturation
n_s = number of binding sites (>1)
n_H = Hill coefficient ~~$n_H \gg 1$~~

$n_H > 1$ positive cooperativity
$n_H = 1$ no cooperativity
$n_H < 1$ negative cooperativity

$[L]$ = free ligand concentration
$\bar{\upsilon}$ = average number of ligands bound per protein

3. KNF model a.k.a. sequential model
Koshland-Nemethy-Filmer (1966)

For a dimer

$$\bar{\upsilon} = \frac{2k_1[L] + 2k_1k_2[L]^2}{1 + 2k_1[L] + k_1k_2[L]^2}$$

where k_1 and k_2 are the intrinsic (microscopic) constants corrected for statistical factors.

The intrinsic constants are related to the macroscopic constants by

$$K_1 = 2k_1 \quad \text{and} \quad K_2 = \tfrac{1}{2}k_2$$

$$K_1K_2 = k_1k_2$$

So the binding equation for the sequential model of a 2-site binding protein is

$$\bar{\nu} = \frac{2k_1[L] + 2k_1k_2[L]^2}{1 + 2k_1[L] + k_1k_2[L]^2} = \frac{K_1[L] + 2K_1K_2[L]^2}{1 + K_1[L] + 2K_1K_2[L]^2}$$

When $k_2 > k_1$, it is positive cooperativity

$k_2 = k_1$, no cooperativity

$k_2 < k_1$, negative cooperativity

4. MWC model a.k.a. concerted ~~note~~ model
Monod-Wyman-Changeux (1965)

Can also be used assess positive cooperative interactions