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**COLLEGE ON BIOPHYSICS:
FROM MOLECULAR GENETICS TO STRUCTURAL BIOLOGY**

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# *MACROMOLECULAR INTERACTIONS: THEORETICAL PRINCIPLES, I*

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# Macromolecular Interactions: Theoretical Principles, I

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# WHY STUDY MACROMOLECULE-LIGAND BINDING?

- Nearly all biological processes proceed or are controlled by processes involving ligand binding reactions.

Types of information accessible from equilibrium binding studies:

A) Number of ligand binding sites (stoichiometry)
  - sites may be discrete or overlapping
  - number of sites may change during interactions among macromolecules

B) Macromolecule-ligand binding constants which can yield thermodynamic information ($\Delta G°, \Delta H°, \Delta S°$)

C) Does ligand bind with cooperativity?

D) Does the ligand effect conformational changes in the macromolecule? Allosteric effects?

E) Is the assembly state of the macromolecule influenced upon ligand binding?

F) Is there ligand release or uptake that accompanies a macromolecular interaction?

G) Does a second ligand (Y) influence the binding of ligand X? (Linkage)

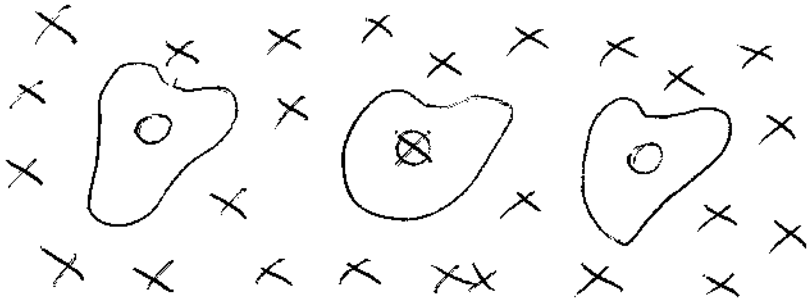
H) How do these interactions relate to the function of the macromolecule?

I) Equilibrium binding information provides the thermodynamic constraints for what is possible and provides the background for kinetic and mechanistic studies.

We will focus first on direct studies of binding of ligand X to a macromolecule M

We wish to know the Average number of ligand bound per macromolecule $\langle x \rangle$ and how this change with ligand concentration

Consider a simple macromolecule that can bind only one ligand



$\langle x \rangle$ The mean value of ligands bound per macromolecule

$$\langle x \rangle = \frac{[\text{Ligands bound}]}{[\text{Total macromolecule}]} = \frac{1 \cdot [MX]}{[MX] + [M]} \qquad (1)$$

$$M + X \overset{K}{\rightleftarrows} MX \qquad K = \frac{[MX]}{[M] \cdot X} \qquad (2)$$

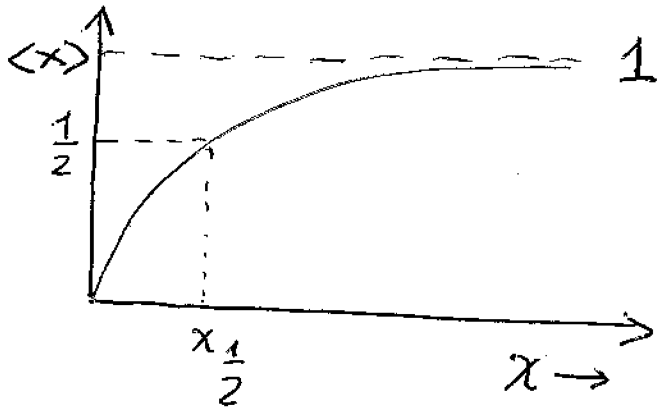
K has units of $(M^{-1})$ association equilibrium constant.

Substituting for [MX] in eq (1)

$$\langle x \rangle = \frac{K[M]x}{K[M]x + [M]} = \left(\frac{KX}{1 + KX}\right)\frac{[M]}{[M]}$$

$$\langle x \rangle = \frac{Kx}{1 + Kx}$$ Describes the binding curve

N.B. $\langle x \rangle$ is independent of the macromolecule concentration



$$x_{1/2} = \frac{1}{K}$$

x is the free ligand concentration or activity, not the total ligand concentration

The reaction can be written as an association or dissociation reaction

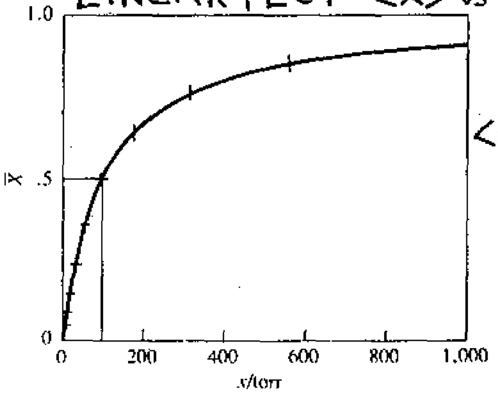
$$M + X \overset{K}{\rightleftarrows} MX \qquad K = \frac{[MX]}{[M][X]} \quad (M^{-1})$$

$$MX \overset{K_d}{\rightleftarrows} M + X \qquad K_d = \frac{[M][X]}{[MX]} \quad (M)$$

$$K = \frac{1}{K_d}$$

# PLOTTING METHODS

## LINEAR PLOT <x> vs x



$$\langle x\rangle = \frac{Kx}{1+Kx}$$

$$x_{\frac{1}{2}} = \frac{1}{K}$$

Figure 2-2.
Single-site X-binding curve showing hyperbolic relation between $\bar{X}$ and ligand activity, $x$. Note that the reciprocal of the half-saturation activity $x_{1/2}$ gives the binding constant $K$, in this case, 0.0100 torr$^{-1}$. The vertical error lines at chosen points represent an error of ±0.02 in $\bar{X}$.

## TITRATION CURVE PLOT <x> vs. log x

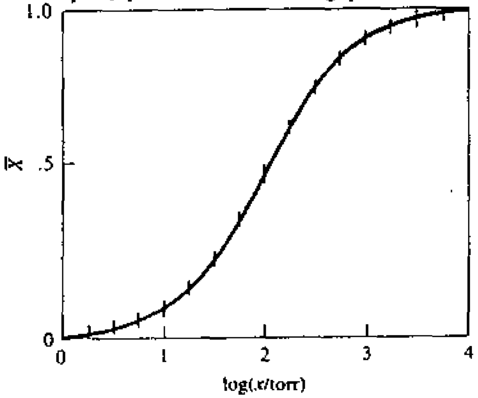
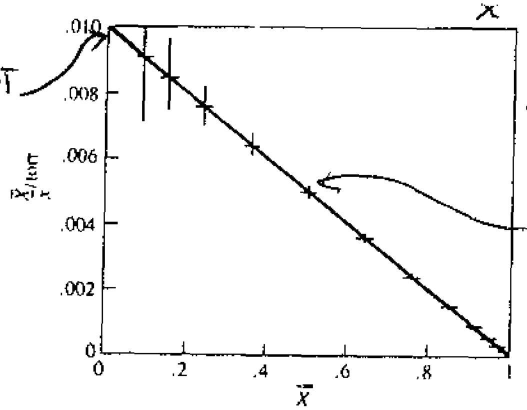


Figure 2-7.
Titration binding curve obtained by plotting $\bar{X}$ versus log $x$ for a single-site reaction where $K = 0.01$. Error of data points is ±0.02 in $\bar{X}$. Compare with plots shown in Figures 2-2 through 2-6.

## SCATCHARD PLOT $\frac{\langle x\rangle}{x}$ vs $\langle x\rangle$



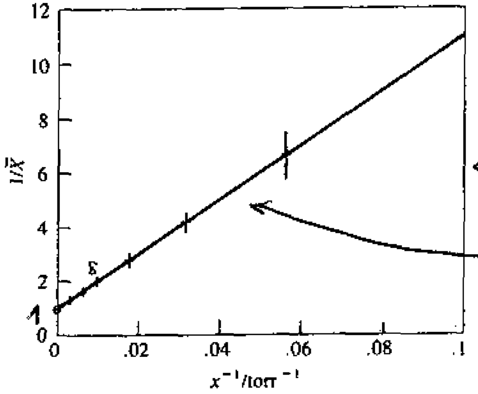
intercept K

$$\frac{\langle x\rangle}{x} = K - K\langle x\rangle$$

SLOPE = − K

Figure 2-4.
The Scatchard plot, $\bar{X}/x$ versus $\bar{X}$, is linear for single-site reaction with a slope of $-K$ and intercepts of 1 and $K$. Error range on points corresponds to fixed error of ±0.02 in $\bar{X}$ as given in Figure 2-2.

## DOUBLE RECIPROCAL PLOT $\frac{1}{\langle x\rangle}$ vs $\frac{1}{x}$



$$\frac{1}{\langle x\rangle} = 1 + \frac{1}{Kx}$$

slope = $\frac{1}{K}$

Figure 2-3.
Double reciprocal plot: $1/\bar{X}$ versus $1/x$. Simulation of same data as given in Figure 2-2. Note how length of error lines differs in this mode of plotting.

The Hill plot $\log\left(\frac{\langle x\rangle}{1-\langle x\rangle}\right)$ vs $\log x$

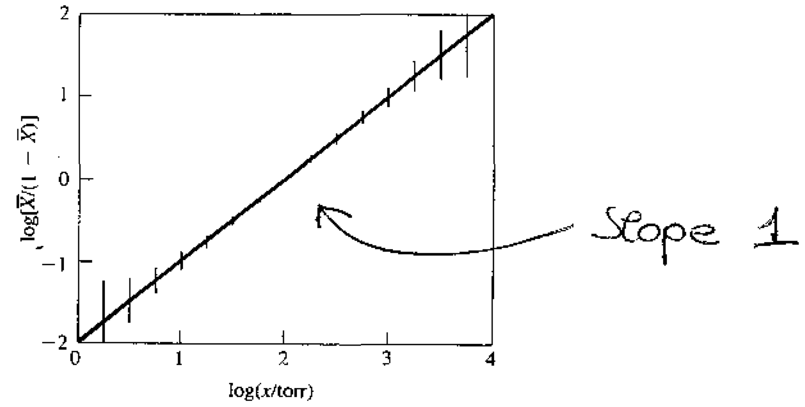


**Figure 2-6.**
Hill plot of a single-site reaction. The logarithm of the ratio of filled ($\bar{X}$) to unfilled sites ($1-\bar{X}$) is plotted against the logarithm of the ligand activity $x$. Note how the error increases greatly in the end regions when the data are plotted in this way.

$$\log\left(\frac{\langle x\rangle}{1-\langle x\rangle}\right) = \log K + \log x$$

$$\frac{\langle x\rangle}{1-\langle x\rangle} = \frac{\text{fraction of sites that are filled}}{\text{fraction of empty sites}}$$

# Macromolecules with multiple ligand binding sites

## 2 general Types of Equilibrium binding "Constants"

1) Average Binding "Constants": Describe the average interaction of a ligand with the multiple sites within the macromolecule

2) Site Binding "Constants": Describes the interaction of a ligand with one particular site on the macromolecule

## 3 Types of Average Binding "Constants"

A) Overall or Stoichiometric ($B_i$)
   - contains statistical factors.

B) Step-wise macroscopic ($K_i$)
   - contains statistical factors.

C) Step-wise microscopic ($k_i$)
   - corrected for statistical factors

A) Overall or Stoichiometric Binding "Constants" ($B_i$)

- Describes the binding of "$i$" ligands to an unligated macromolecule $M_o$

$$M_o + iX \overset{\beta_i}{\rightleftharpoons} MX_i \qquad \beta_i = \frac{[MX_i]}{[M_o][X]^i}$$

Consider a 2 site macromolecule

$$M_o + X \overset{B_1}{\rightleftharpoons} MX \qquad \beta_1 = \frac{[MX]}{[M_o]\,X} \quad (M^{-1})$$

$$M_o + 2X \overset{\beta_2}{\rightleftharpoons} MX_2 \qquad \beta_2 = \frac{[MX_2]}{[M_o]\,X^2} \quad (M^{-2})$$

$\beta_i$ has units of $M^{-i}$

B) Step-wise macroscopic "constants" ($K_i$): Describes binding as the progressive addition of one ligand at a time.

2 site macromolecule

$$M_o + X \overset{K_1}{\rightleftharpoons} MX \qquad K_1 = \frac{[MX]}{[M_o]\,X} \quad (M^{-1})$$

$$MX + X \overset{K_2}{\rightleftharpoons} MX_2 \qquad K_2 = \frac{[MX_2]}{[MX]\,X} \quad (M^{-1})$$

All $K_i$ have units of $M^{-1}$

Note:

$$\boxed{\begin{aligned} \beta_1 &= K_1 \\ \beta_2 &= K_1 K_2 \\ \beta_i &= \prod_{i=1}^{n} K_i \end{aligned}}$$

c) Step-wise microscopic "constants" ($k_i$)

- These describe the same reactions as do the step-wise macroscopic "constants", but have been corrected for the "statistical factors" that describe the # of distinguishable ways to place "i"th ligand on the macromolecule

These binding constants reflect the average binding properties of the ligand to the sites, in the absence of the statistical factors.

2 site macromolecule

$$M_0 + 2X \overset{K_1}{\rightleftharpoons} MX + X \overset{K_2}{\rightleftharpoons} MX_2$$

$$K_1 = 2k_1 \qquad K_2 = \frac{k_2}{2}$$

statistical factors

In step 1 : $K_1$ : There are 2 ways to form MX from one $M_0$

or


statistical factor = 2

In step 2 : $K_2$ : only 1 way to form $MX_2$ for each of the 2 MX

statistical factor = $\frac{1}{2}$

$$\text{statistical factor} = \frac{\#\text{ of configuration with "}i\text{" bound ligands}}{\#\text{ of configuration with "}i-1\text{" bound ligands}}$$

Average number of ligands bound per macromolecule $\langle x \rangle$ :

$$\langle x \rangle = \frac{X_{bound}}{M_{TOTAL}} = \frac{\sum_{i=0}^{n} i[M_i]}{\sum_{i=0}^{n}[M_i]} = \frac{\sum_{i=0}^{n} i\beta_i [M_0] x^i}{\sum_{i=0}^{n} \beta_i [M_0] x^i}$$

$$\beta_i = \frac{[M_i]}{[M_0]x^i}$$

$$= \frac{\sum_{i=0}^{n} i\beta_i x^i}{\sum_{i=0}^{n} \beta_i x^i}$$

$\langle x \rangle$ is independent of the macromolecule concentration

$\langle x \rangle$ depends only on x, the free ligand concentration (activity)

For a macromolecule with a single ligand binding site $n = 1$

$$\langle x \rangle = \frac{\beta_1 x}{1 + \beta_1 x} = \frac{Kx}{1 + Kx} = \frac{[MX]}{[M_0] + [MX]}$$

For $n = 2$ (Two sites macromolecule)

$$\langle x \rangle = \frac{\beta_1 x + 2\beta_2 x^2}{1 + \beta_1 x + \beta_2 x^2} = \frac{[MX] + 2[MX_2]}{[M_0] + [MX] + [MX_2]}$$

For a n sites macromolecules

$$\langle x \rangle = \frac{\beta_1 x + 2\beta_2 x^2 + \dots + i\beta_i x^i + \dots + n\beta_n x^n}{1 + \beta_1 x + \beta_2 x^2 + \dots + \beta_i x^i + \dots + \beta_n x^n}$$ Adair equation

The denominator of the Adair equation has a special significance. It is called the <u>Binding Partition Function</u> or Binding Polinomial

$$P = \sum_{i=0}^{n} \beta_i x^i = 1 + \beta_1 x + \beta_2 x^2 + \dots + \beta_i x^i + \dots + \beta_n x^n$$

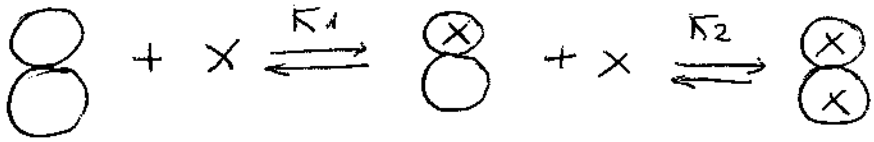
$$P = \frac{[M_0] + [M_1] + [M_2] + \dots + [M_i] + \dots + [M_n]}{[M_0]}$$

where $M_i$ = macromolecule with "i" bound ligands.

P = sum of all macromolecule species concentrations diveded by concentration of macromolecule ($M_0$)
($M_0$ is used as the reference state)

Consider a Two-site macromolecule

Three possible configuration



$$M + X \overset{K_1}{\rightleftarrows} MX \quad ; \quad K_1 = \frac{[MX]}{[M]\cdot X} = 2k_1 \quad , \quad K_1 = \beta_1$$

$$MX + X \overset{K_2}{\rightleftarrows} MX_2 \quad ; \quad K_2 = \frac{[MX_2]}{[MX]\cdot X} = \frac{k_2}{2} \quad ; \quad K_1K_2 = \beta$$

$$\langle x \rangle = \frac{[MX] + 2[MX_2]}{[M_0] + [MX] + [MX_2]} = \frac{\beta_1 X + 2\beta_2 X^2}{1 + \beta_1 X + \beta_2 X^2} = \frac{K_1 X + 2K_1K_2X^2}{1 + K_1X + K_1K_2X^2} = \frac{2k_1X + 2k_1k_2X^2}{1 + 2k_1X + k_1k_2X^2}$$

At half-saturation of the macromolecule, $\langle x \rangle = 1$

$x_{\frac{1}{2}}$ = free ligand concentration at half-saturation

$$x_{\frac{1}{2}} = \frac{1}{\sqrt{k_1 k_2}}$$

## 3 possible cases

A) Non-cooperative $K_1 = K_2 = K$ (Independent Identical sites)

$$\langle x \rangle = \frac{2Kx + 2K^2x^2}{1 + 2Kx + K^2x^2} = \frac{2Kx(1+Kx)}{(1+Kx)^2}$$

$$\langle x \rangle = \frac{2Kx}{1+Kx} \qquad x_{1/2} = \frac{1}{\sqrt{K_1K_2}} = \frac{1}{K}$$

This is the same result of a single site macromolecule

If $K_1 \neq K_2$, then binding is cooperative and the sites are linked

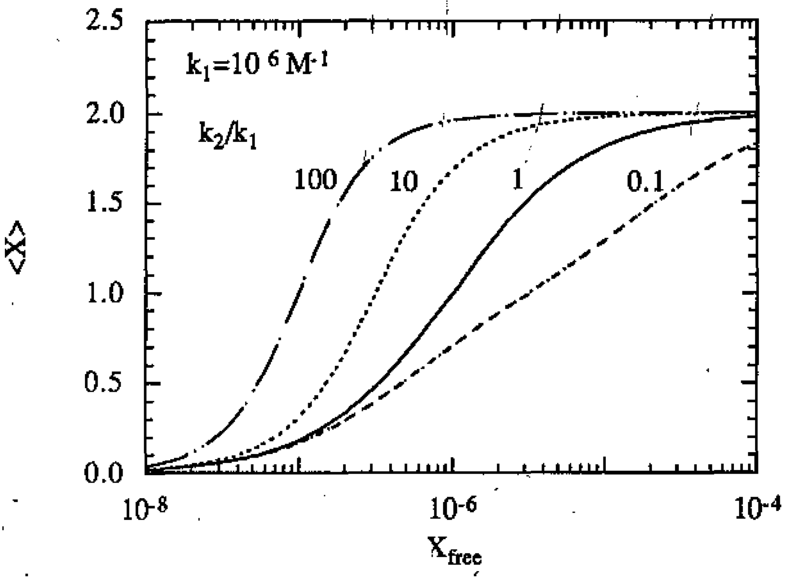
B) Positive Cooperativity : $K_1 < K_2$

C) Negative Cooperativity : $K_1 > K_2$

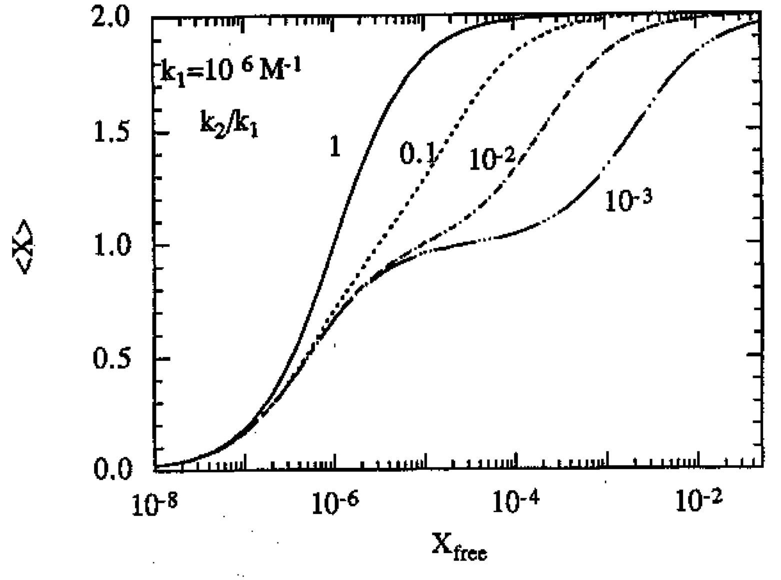
IMP: Need to compare step-wise microscopic binding constants $K_1$ and $K_2$ since these do not contain the statistical factors reflecting the # of distinct configurations

## 2-site macromolecule

Titration (+)

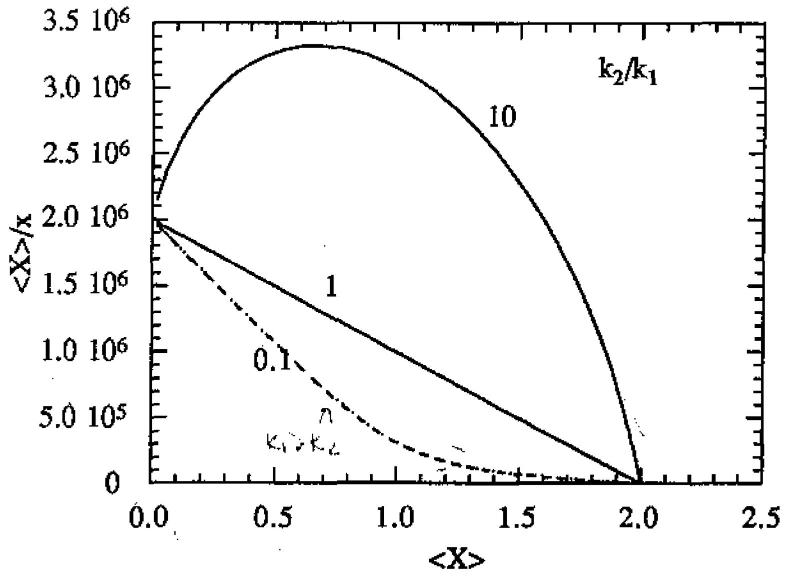


Titration (–)

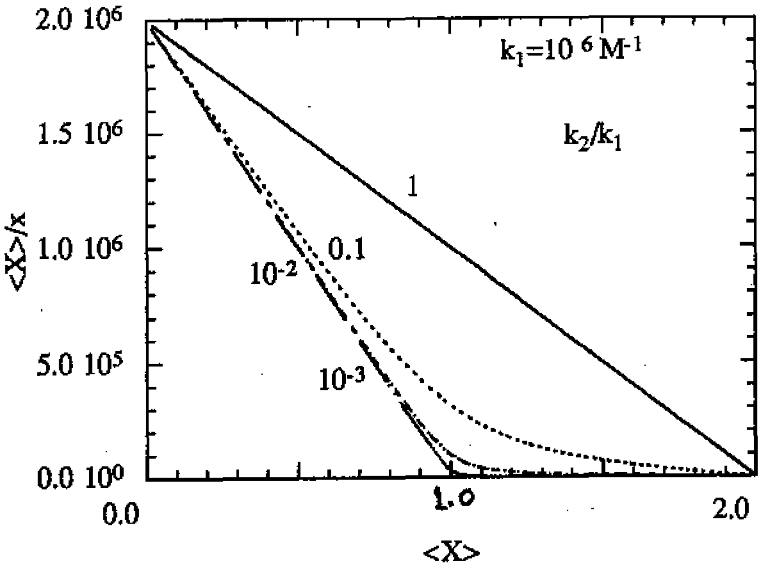


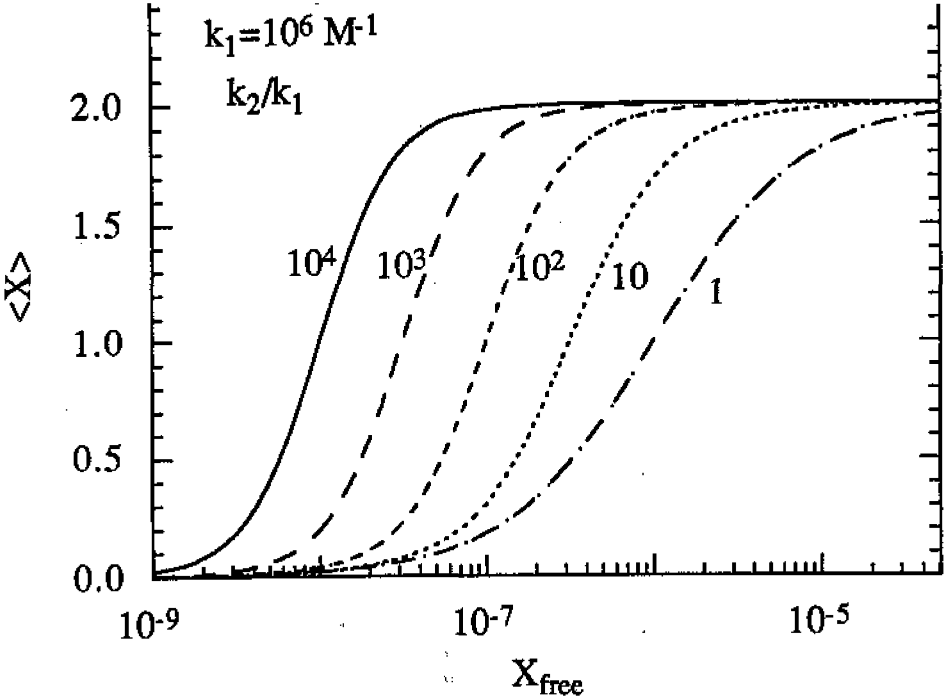
$$\langle X\rangle = \frac{2k_1 x + 2k_1 k_2 x^2}{1 + 2k_1 x + k_1 k_2 x^2}$$

Scatchard (+)



Scatchard (–)

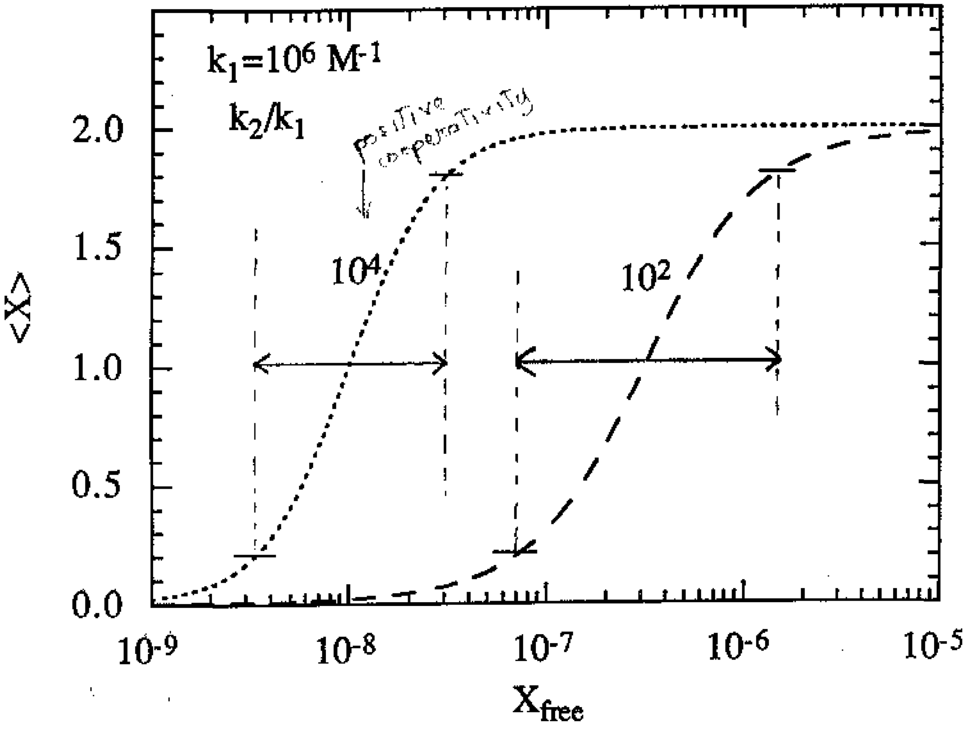


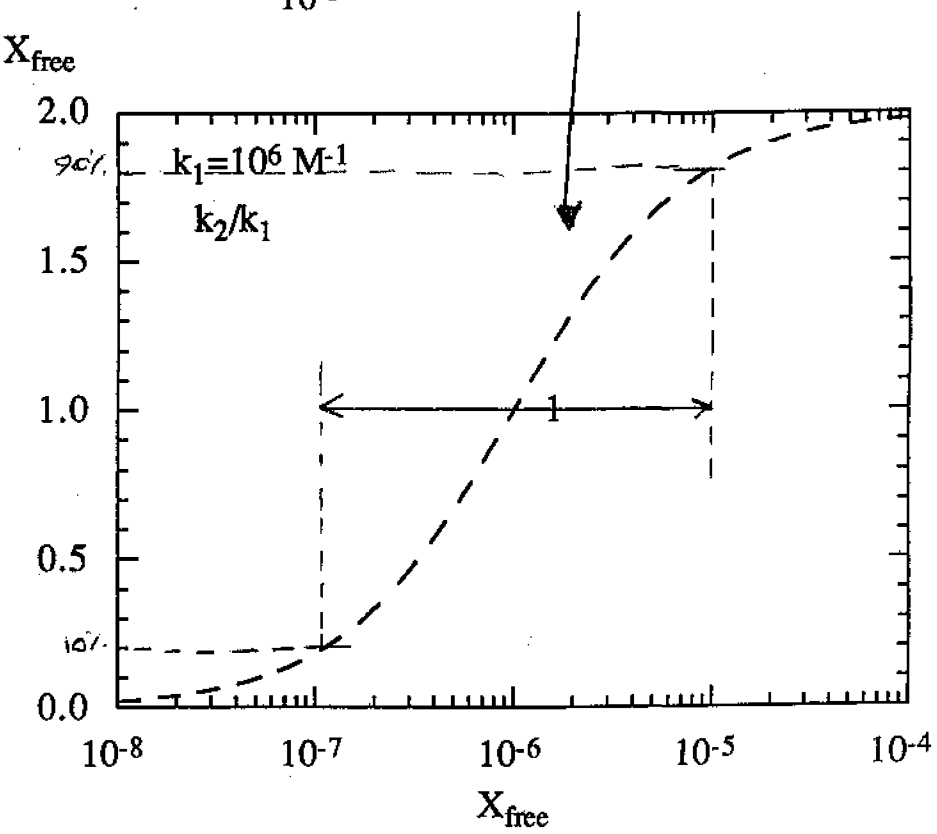


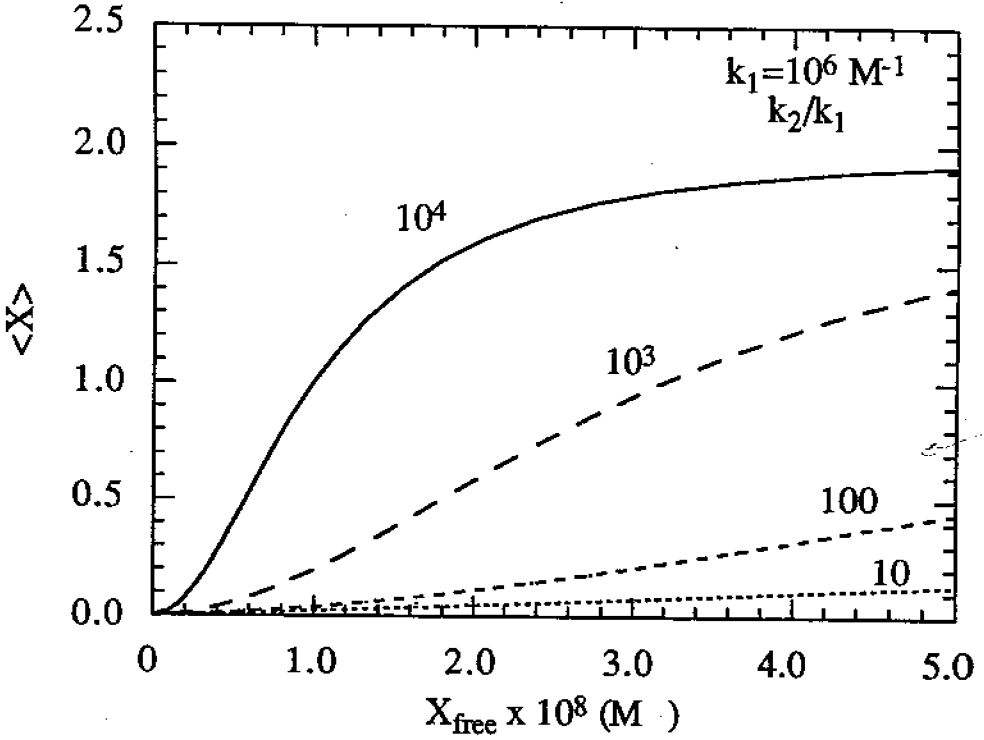
For independent and identical binding

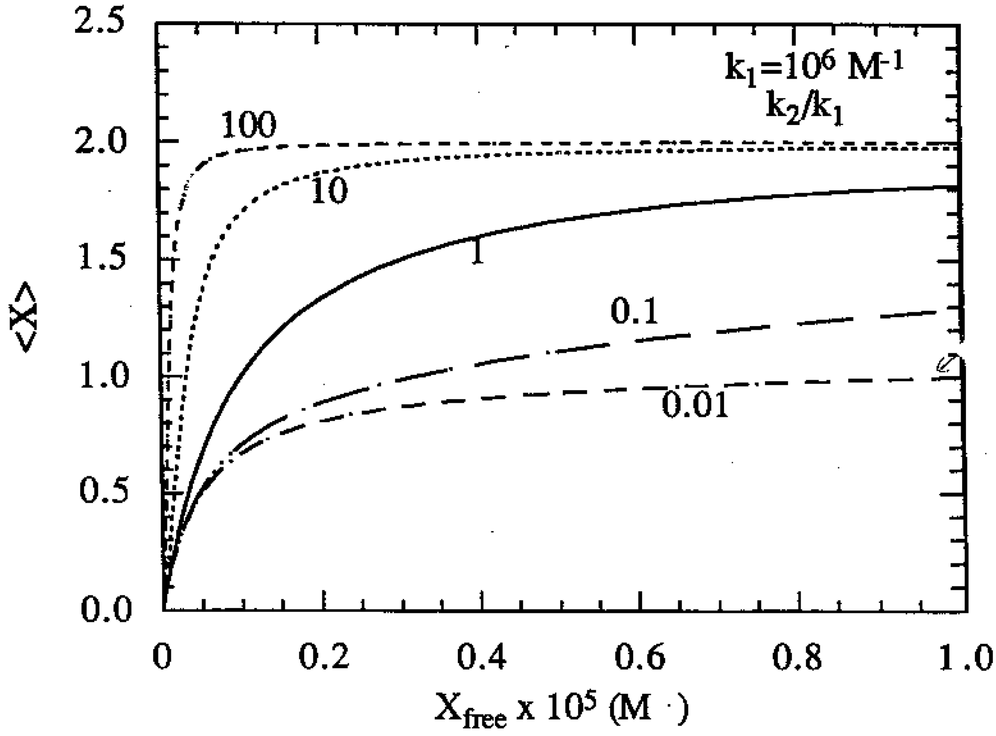
$$\log\left(\frac{[X]_{90\%\,SAT}}{[X]_{10\%\,SAT}}\right) = 1.91$$

X spans 2 log units









← "apparent" plateau

The Hill plot for a 2 site macromolecule

$$\langle x\rangle = \frac{[MX] + 2[MX_2]}{[M_0] + [MX] + [MX_2]} = \frac{2K_1X + 2K_1K_2X^2}{1 + 2K_1X + K_1K_2X^2}$$

$\boxed{\text{Hill plot}}$: $\ln\left(\frac{\langle x\rangle}{2-\langle x\rangle}\right)$ vs $\ln X$

$$\boxed{n_H = \text{Hill coefficient is the slope when half of the sites are filled. } (\langle x\rangle = 1)}$$

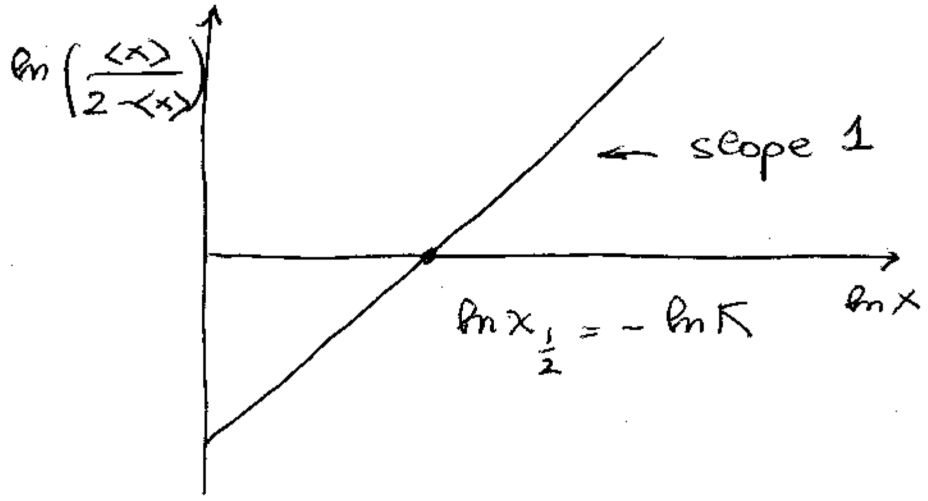
$n_H = 1$ NO cooperativity

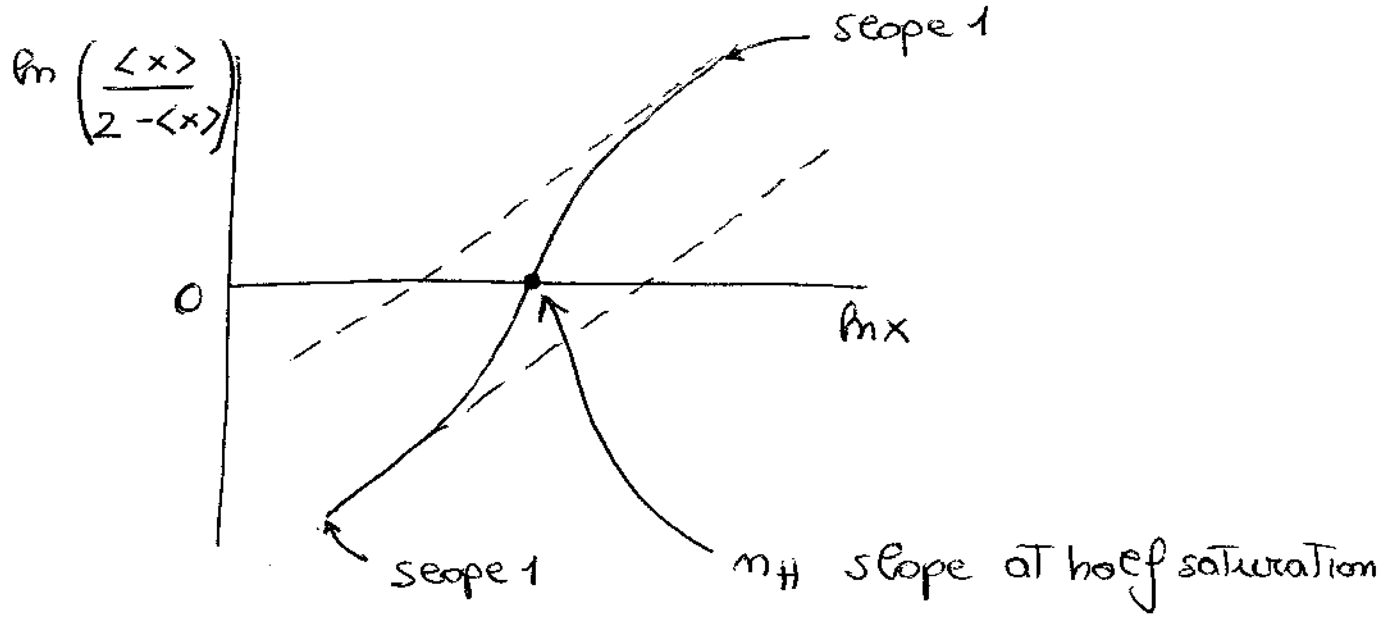
$n_H > 1$ positive cooperativity

$n_H < 1$ negative cooperativity

If NO cooperativity $K_1 = K_2 \Rightarrow \langle x\rangle = \frac{2KX}{1+KX}$

$$\frac{\langle x\rangle}{2-\langle x\rangle} = KX \Rightarrow \ln\left(\frac{\langle x\rangle}{2-\langle x\rangle}\right) = 1\ln K + \ln X$$





$$\frac{\langle x\rangle}{2-\langle x\rangle} = \frac{2K_1 x + 2K_1K_2 x}{1+2K_1 x}$$

The limiting slopes at low x and high x approach unity reflecting binding of the first and the last ligands respectively

For a "n" sites macromolecule : $\frac{\langle x\rangle}{n-\langle x\rangle}$

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Book: Jeffries Wyman and Stanley J. Gill
"University Science Book"
Title : "Binding and Linkage"