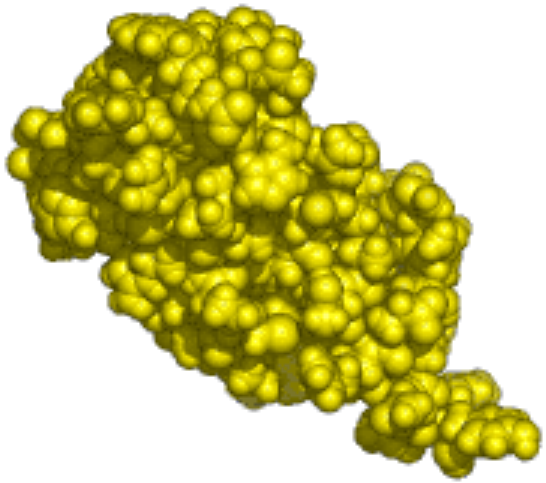
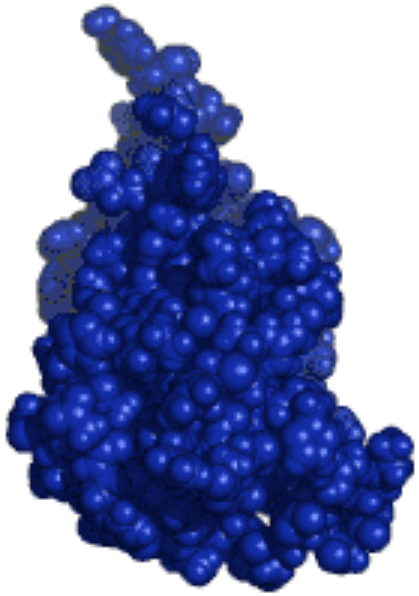
Binding



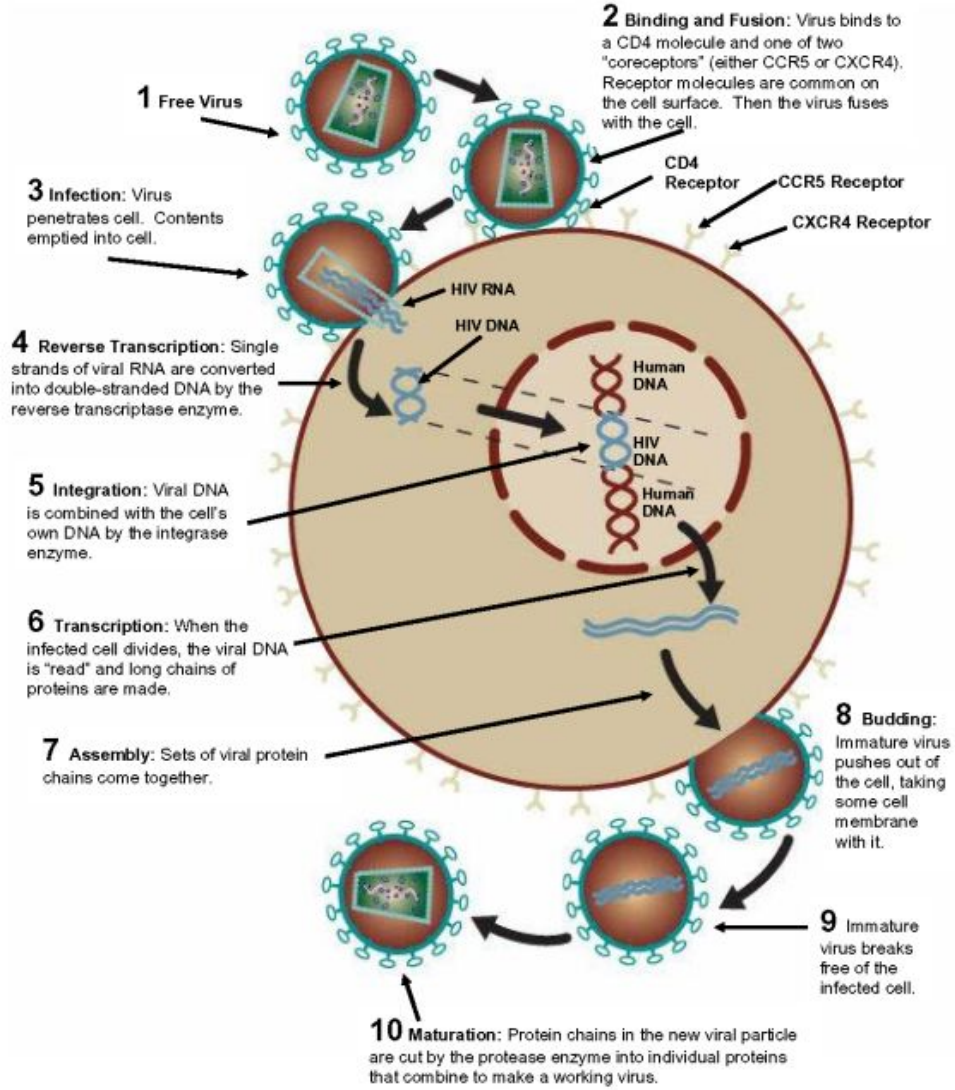


A substance is not effective unless it is linked to another."

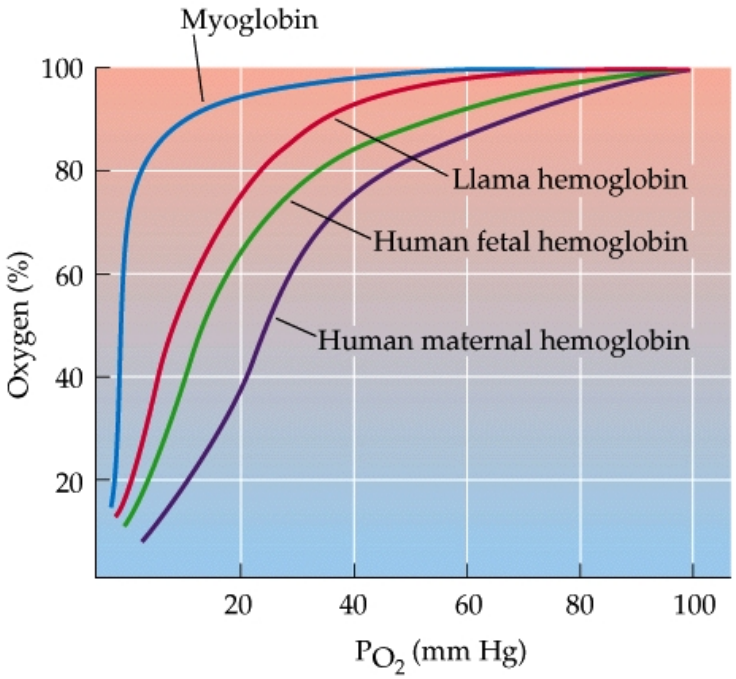
Paul Ehrlich

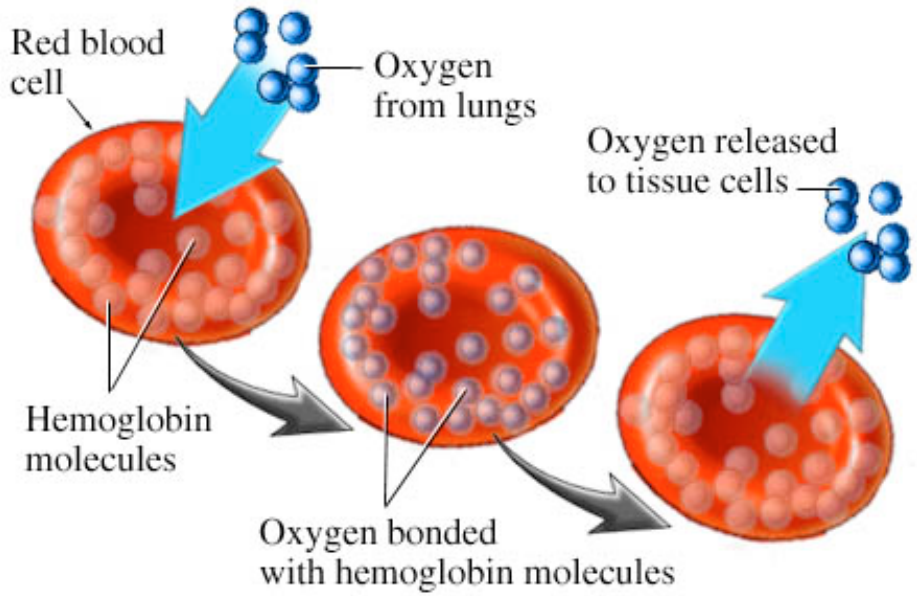
The Role of Binding in the HIV Life Cycle

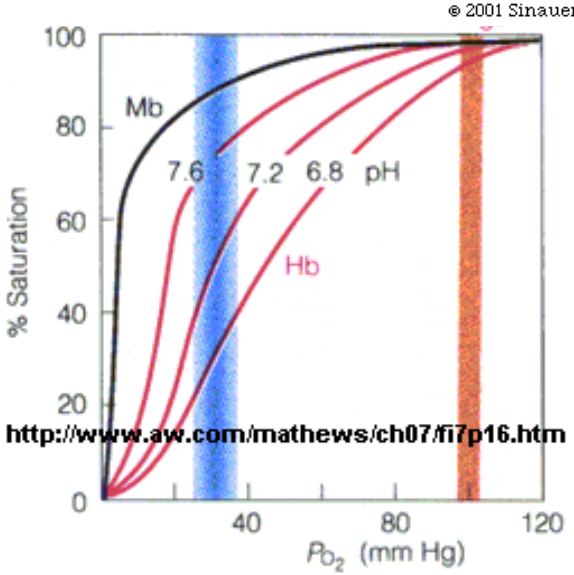
One of the first processes in the infection process is the binding of the virus to the host cell.



Hemoglobin Binding Curves

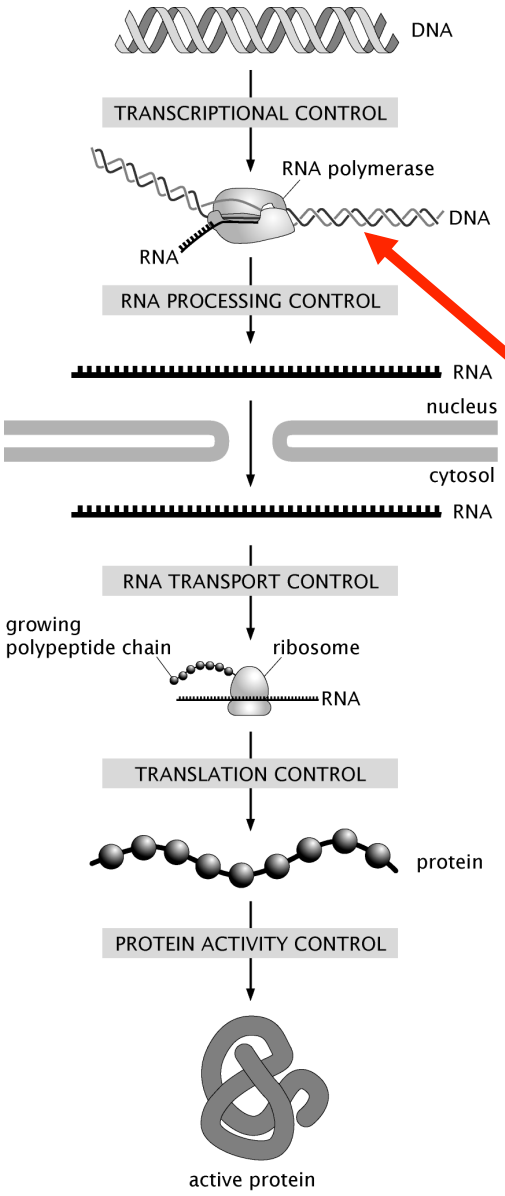




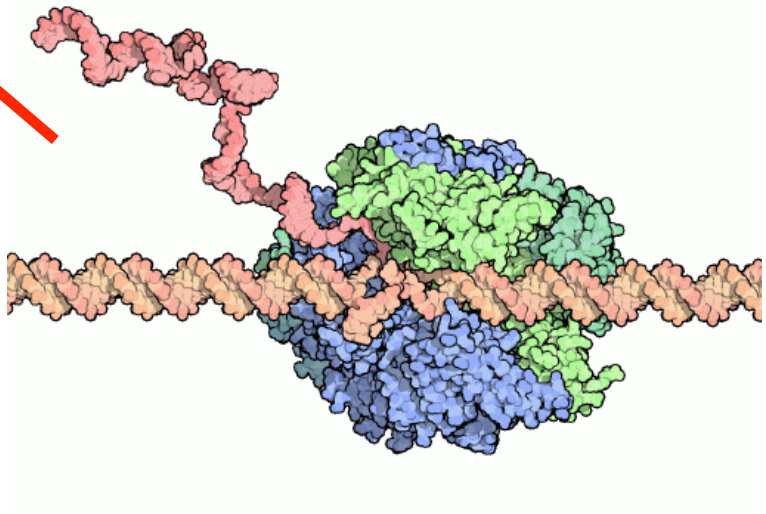


- *``A substance is not effective unless it is linked to another." - Paul Ehrlich*
- *O_2 binding curves*

Transcriptional Regulation



- ***Regulation takes place very far upstream. In particular, the "decision" is made whether or not to produce mRNA.***
- ***Question: What are the molecules that mediate this control?***
- ***Molecular binding events on DNA are a key mechanism of control.***



Antibody-Antigen Binding

The Immune System

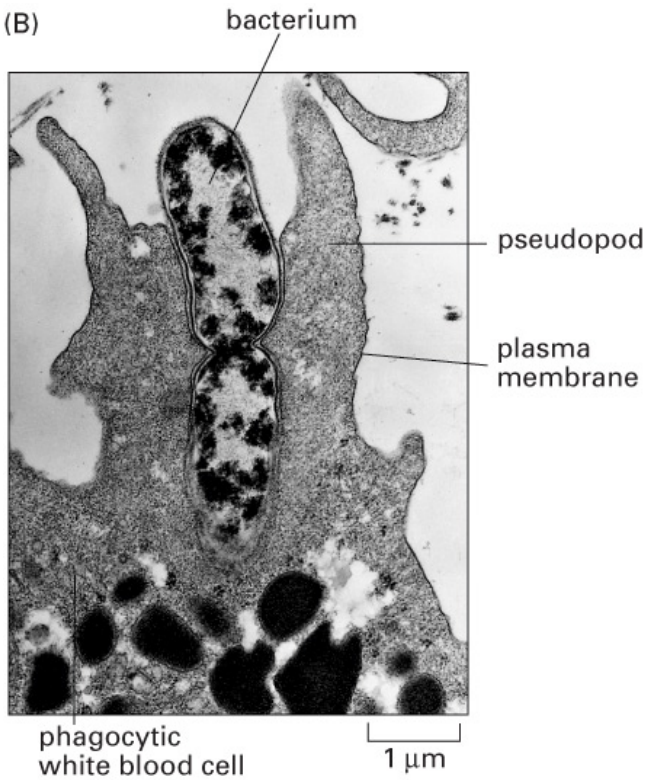
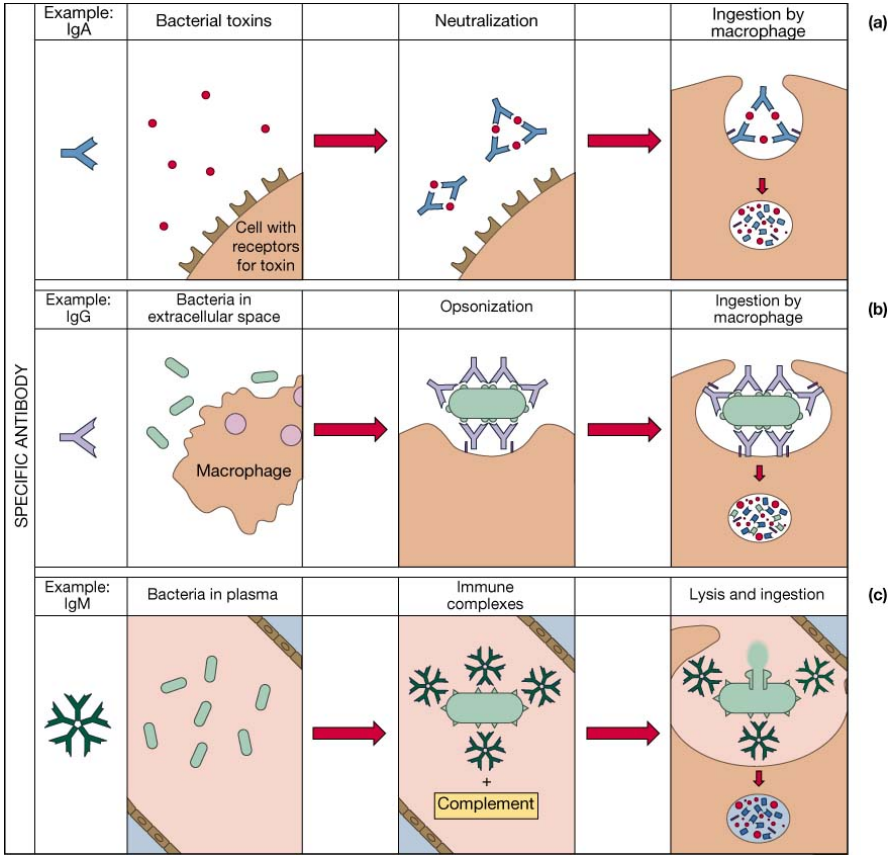


Figure 24–24 part 2 of 2. Molecular Biology of the Cell, 4th Edition.



Molecular recognition mediated by binding reactions is at the centre of immune response.

Energetic Term	Contribution	Physical Description
Conformational Entropy	-1,500 kJ/mol	Disorder of main and side chain
Hydrophobic Interactions	+ 1,255 kJ/mol	Ordering of water molecules
Van der Waals (dipole)	+ 170 kJ/mol	Breakage of van der Waals forces in protein core
Hydrogen bonds	+ 45 kJ/mol	Breakage of mainchain and sidechain hydrogen bonds[1].
Electrostatic Interactions	+ 5 kJ/mol	Loss of favorable charge-charge interactions
Net Sum	**+ 25 kJ/mol**	

An estimate of the contribution of the various factors to ΔG^o for binding of a ~ 30 residue proteins (T = 300 ^{o}K).

A typical form in which energy of interactions between two proteins or protein and small molecule can be written

$$E_{tot} = \underbrace{\sum q\phi_{el}}_{\text{Ionic pairs + H-bonding}} + \underbrace{A\phi_{steric} + B\phi_{disp}}_{\text{Van der Waals}} + \underbrace{E_{desolv}}_{\text{removal of water from the contact}}$$

Evaluation of binding

$$A + B \leftrightarrow AB$$

Thermodynamic properties

ΔH enthalpy change

ΔS entropy change

ΔC heat capacity change

Affinity – strength of binding

K_A (affinity constant) or K_D (dissociation constant)

$K_D = 1/K_A$

Kinetics

k_{ass} or k_{on} association rate constant or on rate

k_{diss} or k_{off} dissociation rate constant or off rate

Mechanical properties *(e.g. unbinding force, elasticity)*

Affinity

$$A + B \xrightarrow{K_A} AB$$

It is measured with the standard free energy of binding ΔG^0.

$$\Delta G^0 = \Delta H^0 - T\Delta S^0$$

$\Delta G^0 < 0$ – binding is favoured.

ΔG^o is the standard state which assumes all components are at the standard state concentration of 1 M (mol.L^{-1}).

$$\Delta G^0 = -RT \ln K_A$$

Association constant K_A

$$K_A = \frac{[AB]}{[A][B]} = e^{-\frac{\Delta G_{BA}}{RT}}$$

- The units (M^{-1})
- The ratio of [products] versus [reactants] at equilibrium.
- Higher affinity = higher K_A
- Depends on B concentration

Dissociation constant K_D

$$A + B \underset{K_D}{\leftarrow} AB$$

$$K_D = \frac{[A][B]}{[AB]}$$

- Concentration of A at which half of B is bound ([B]=[AB])
- Units are M
- Higher affinity = lower K_D

The complex lifetime – *is independent of the concentration, it depends only on the bond energy (K_D is described by k_{-1}).*

Allosteric activators of enzymes e. g. NAD:

K_D = ***0.1 μM to 0.1 mM.***

Antibody-antigen interaction

K_D = ***0.1 mM to 0.0001 pM.***

Types of binding

all binding sites are
equivalent and independent

cooperativity ↙ ↘ ***heterogeneity***

all binding sites are
equivalent and not independent

all binding sites are
independent but not equivalent

heterogeneity ↘ ↙ ***cooperativity***

all binding sites are
not equivalent and not independent

simple

difficult

very difficult

Average number of ligand molecules bound to macromolecule:

$$\bar{n} = \frac{\text{concentration of A bound to B}}{\text{Total concentration of B}}$$

$$\theta = \frac{\bar{n}}{n} \qquad \begin{array}{l} 0 \le \bar{n} \le n \\ 0 \le \theta \le 1 \end{array}$$

Fractional saturation

Single binding site

$$A + B \Leftrightarrow AB$$

$$0 \le \bar{n} \le 1 \qquad \bar{n} = \theta$$

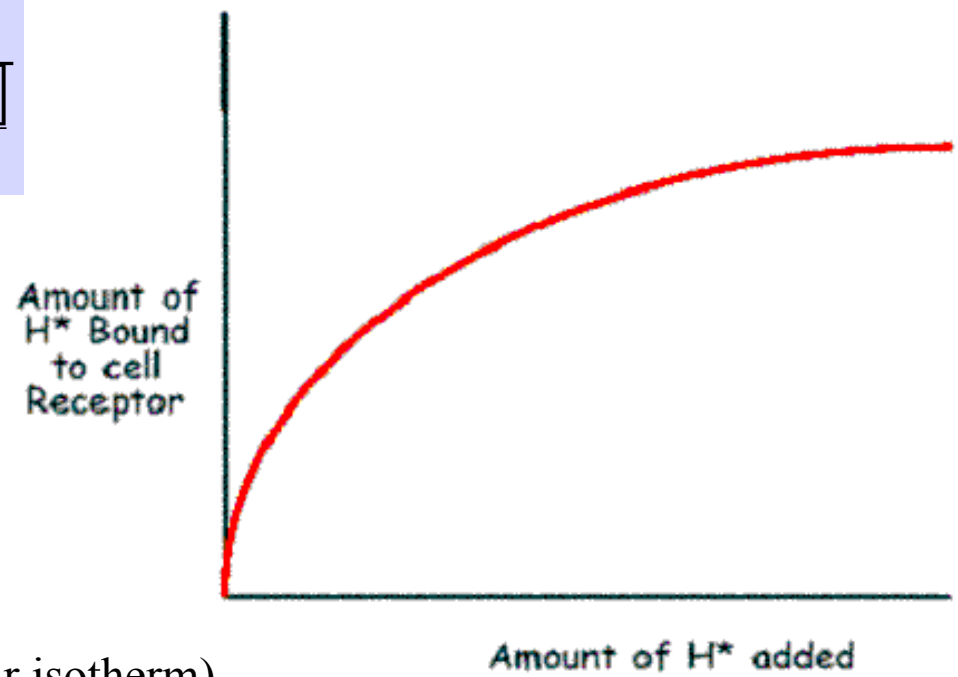
$$\bar{n} = \frac{[AB]}{[B]+[AB]}$$

$$K_D = \frac{[A][B]}{[AB]} \Rightarrow [AB] = \frac{[A][B]}{K_D}$$

$$\bar{n} = \frac{[AB]}{[B]+[AB]} = \frac{\frac{[A][B]}{K_d}}{[B]+\frac{[A][B]}{K_d}}$$

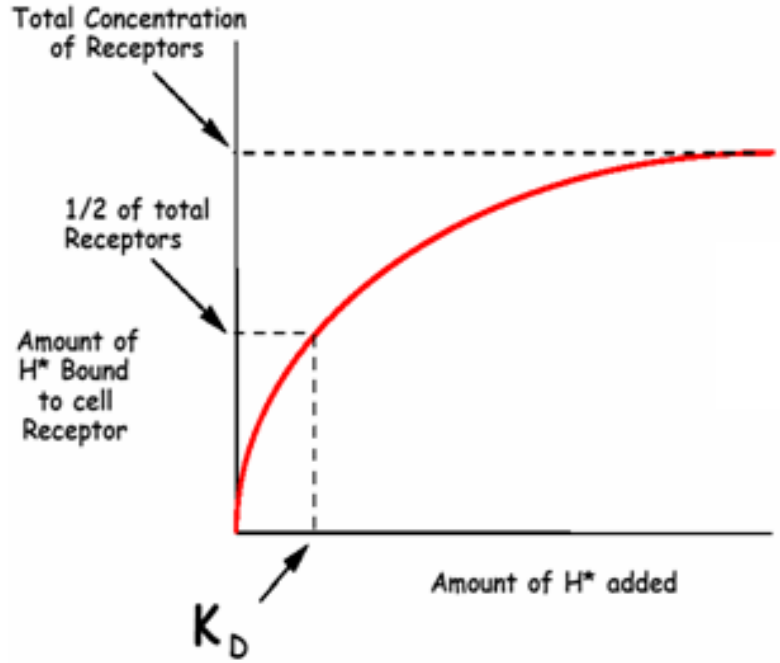
$$\bar{n} = \frac{[A]}{K_d + [A]}$$

'titration curve'



(Langmuir isotherm)

K_D can be obtain directly from the binding curve.



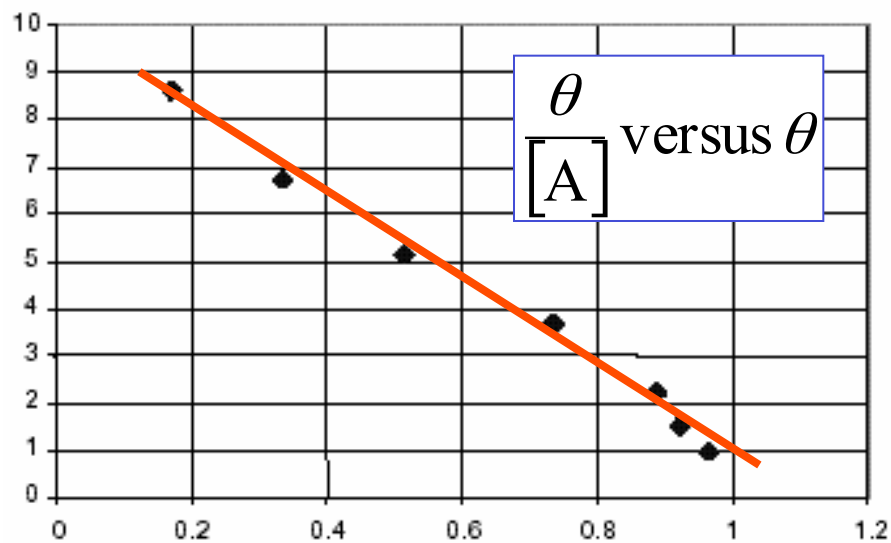
$$\theta = \frac{[A]}{K_d + [A]} \Rightarrow \frac{\theta}{[A]} = \frac{1}{K_d + [A]}$$

$$\frac{1}{K_d + [A]} = \frac{1}{K_d} - \frac{1}{K_d} + \frac{1}{K_d + [A]} =$$

$$\frac{1}{K_d} - \frac{K_d + [A] - K_d}{K_d(K_d + A)} = \frac{1}{K_d} - \frac{[A]}{K_d(K_d + A)} \Rightarrow$$

Scatchard plot

$$\boxed{\frac{\theta}{[A]} = \frac{1}{K_d} - \frac{\theta}{K_d}}$$

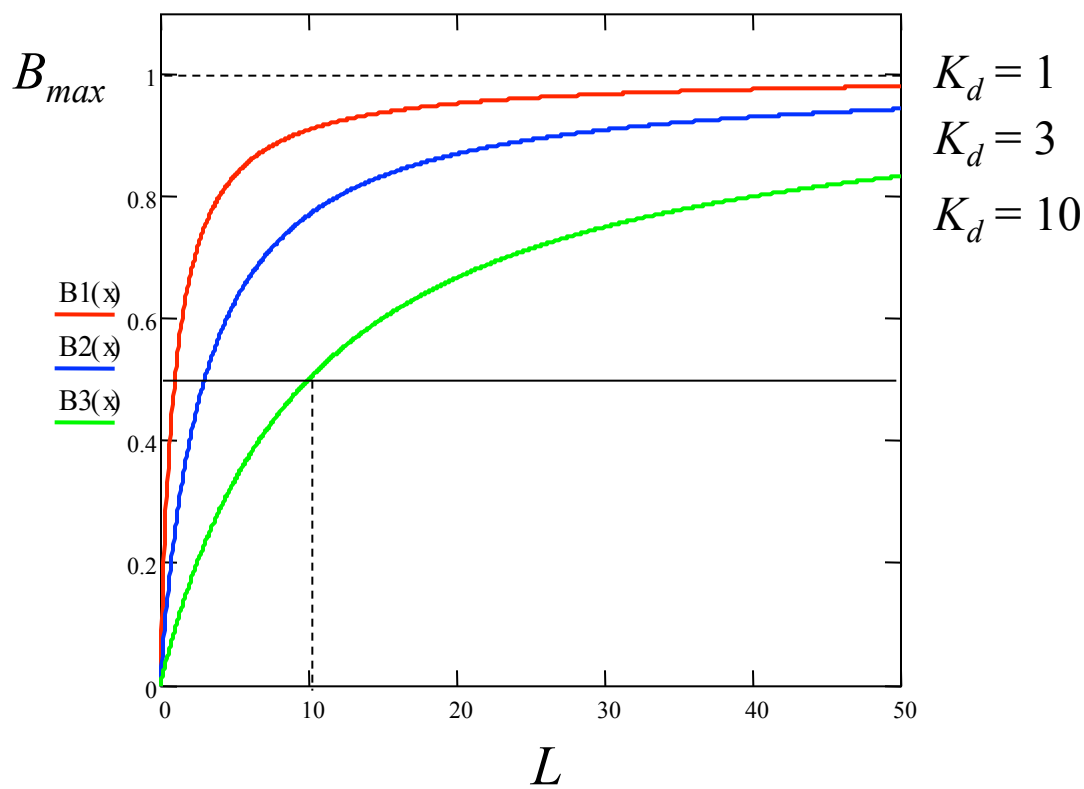


Straight line

Receptor occupancy is a hyperbolic function of [L] (Langmuir adsorption isotherm)

$$\theta = \frac{[L]}{K_d + [L]}$$

K_d has the dimension of concentration and should be measured in the same units as L (M).



Note that for a shallow curve it is hard to say where it saturates

Binding to n identical and independent binding sites

Average number of ligands bound per protein:

$$\bar{n} = n\theta = \frac{n[A]}{K_d + [A]}$$

$$\theta = \frac{\bar{n}}{n}$$

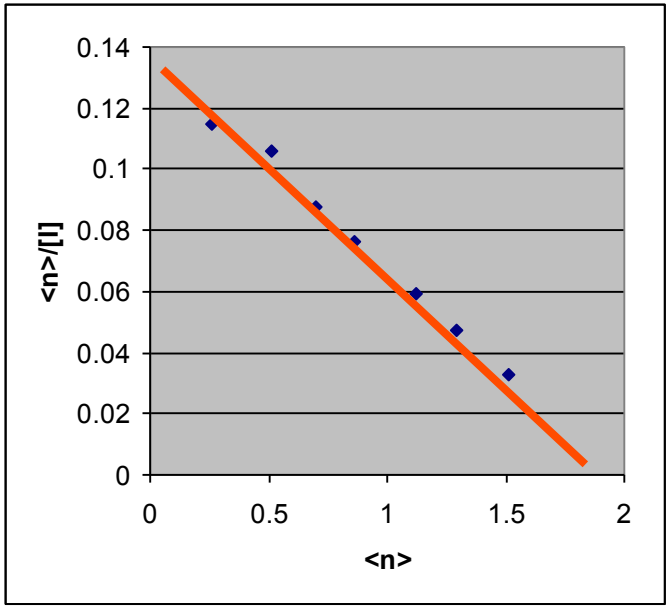
- *Single ligand species.*
- *All sites have the same K_d.*
- *Sites are independent.*
- *Fractional saturation is identical.*

$$\theta = \frac{[A]}{K_d + [A]}$$

Determination of the number of sites

$$\frac{\bar{n}}{[A]} = \frac{n}{K_D} - \frac{\bar{n}}{K_D}$$

Scatchard plot



$$y = ax + b$$

$$a = -\frac{1}{K_D} \qquad b = \frac{n}{K_D}$$

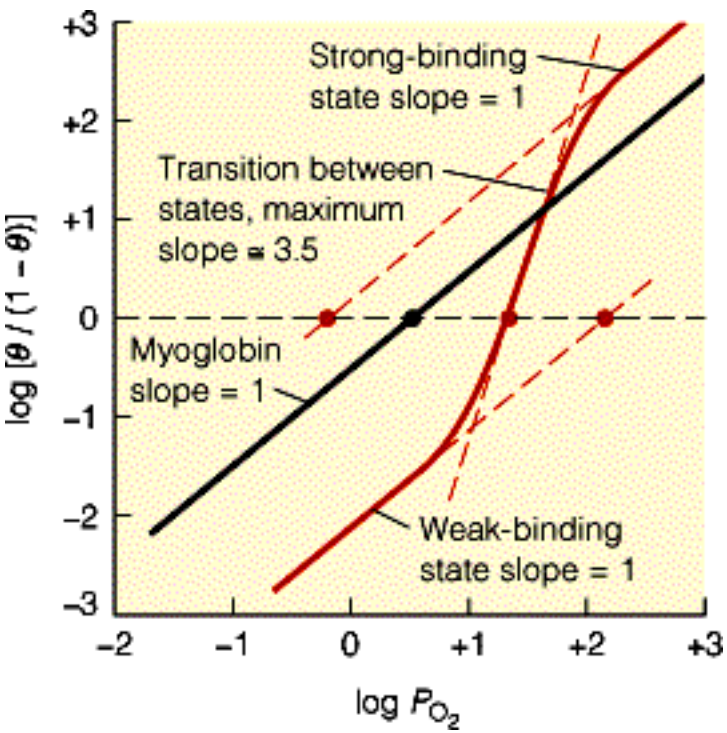
The Hill coefficient – h, a measure of cooperativity

$$\bar{n} = \frac{n[\mathrm{A}]^h}{\mathrm{K}_d + [\mathrm{A}]^h}$$

Hill plot

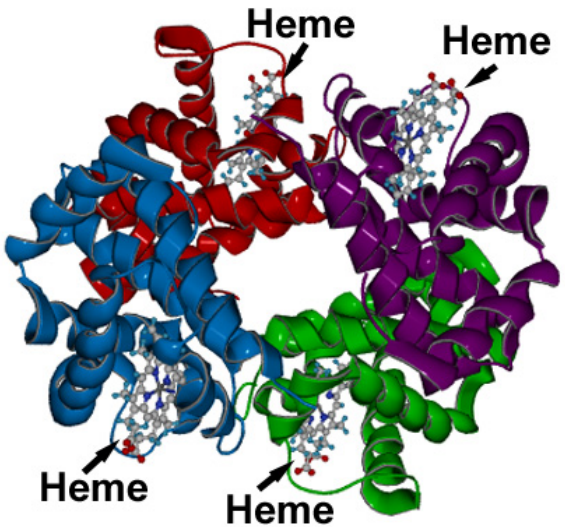
$$\theta = \frac{\bar{n}}{n}$$

$$\log\frac{\bar{n}}{n-\bar{n}} = \log\frac{\theta}{1-\theta} = h\log[A] - \log \mathrm{K}_d$$



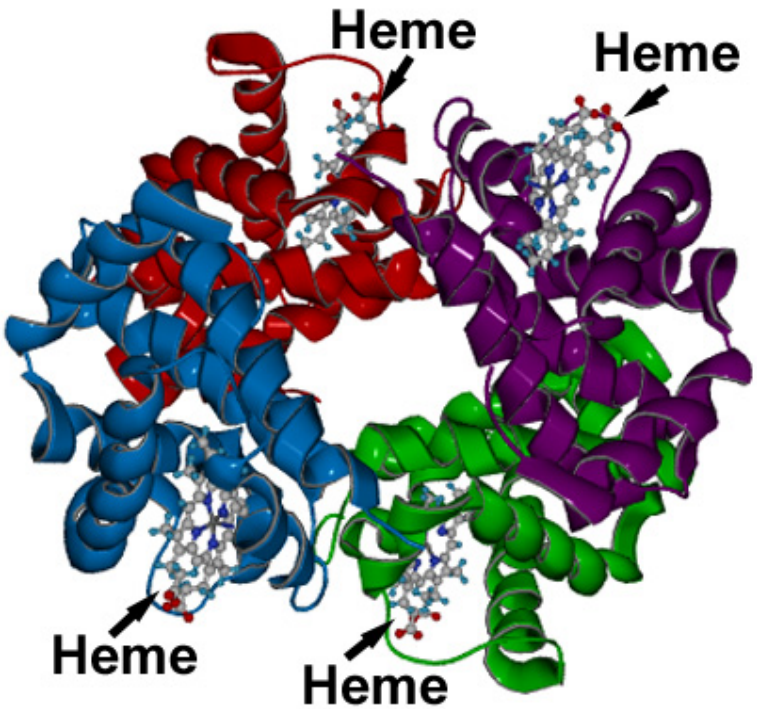
Intercept at log{θ/(1- θ)} = 0 gives logK$_D$.

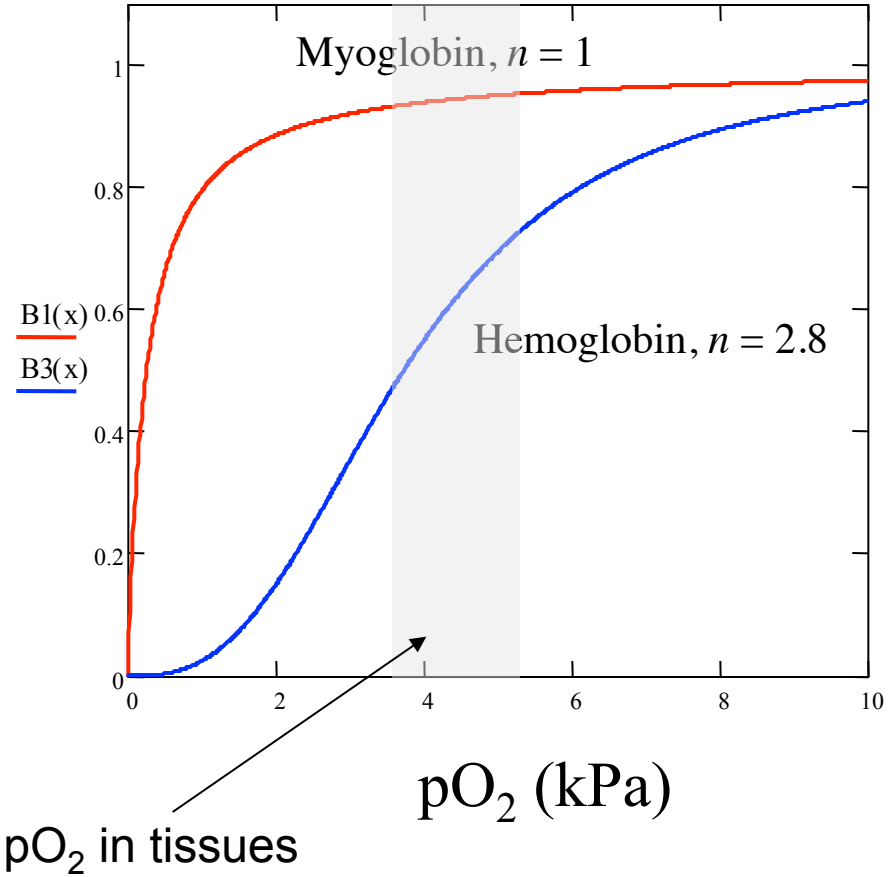
Hill plots of oxygen binding for myoglobin and hemoglobin.



Hemoglobin vs. Myoglobin

$$\theta_n = \frac{[L]^n}{K_n + [L]^n}$$





Hill Plot

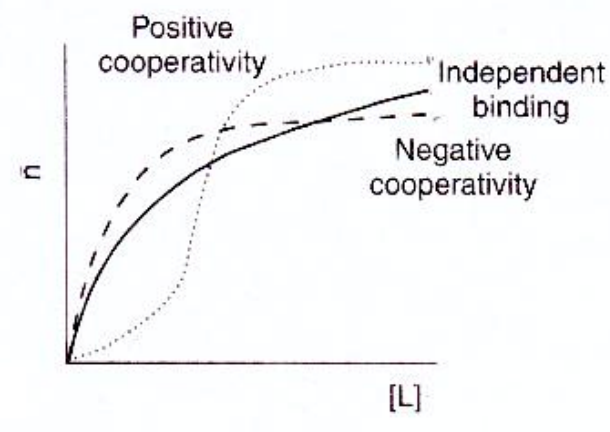
Slope at log{θ/(1- θ)} = 0 is the Hill coefficient, h.

$n_h = 1$ for non-cooperative binding

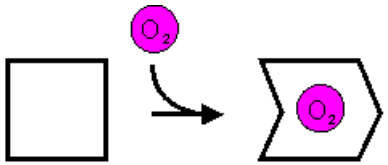
$n_h < 1$ for negative cooperativity

$n_h > 1$ for positive cooperativity

$n_h = n$ (number of sites) for infinitely positive cooperativity.

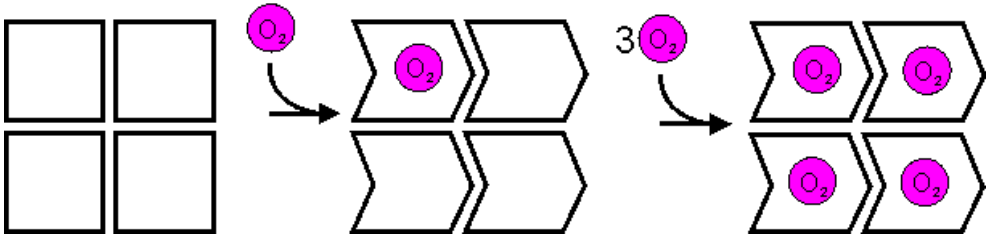


independent binding



$$\theta = \frac{x}{k_d + x}$$

cooperative binding



$$\theta = \frac{x^n}{k_d + x^n}$$

n – Hill coefficient