Biochemistry II: Binding of ligands to a macromolecule (or the secret of life itself...)

- http://www.kip.uni-heidelberg.de/chromcon/publications/pdf-files/Rippe_Futura_97.pdf
- **Principles of Physical Biochemistry, van Holde, Johnson & Ho, 1998.**
- **Slides available at**
- http://www.kip.uni-heidelberg.de/chromcon/teaching/index_teaching.html

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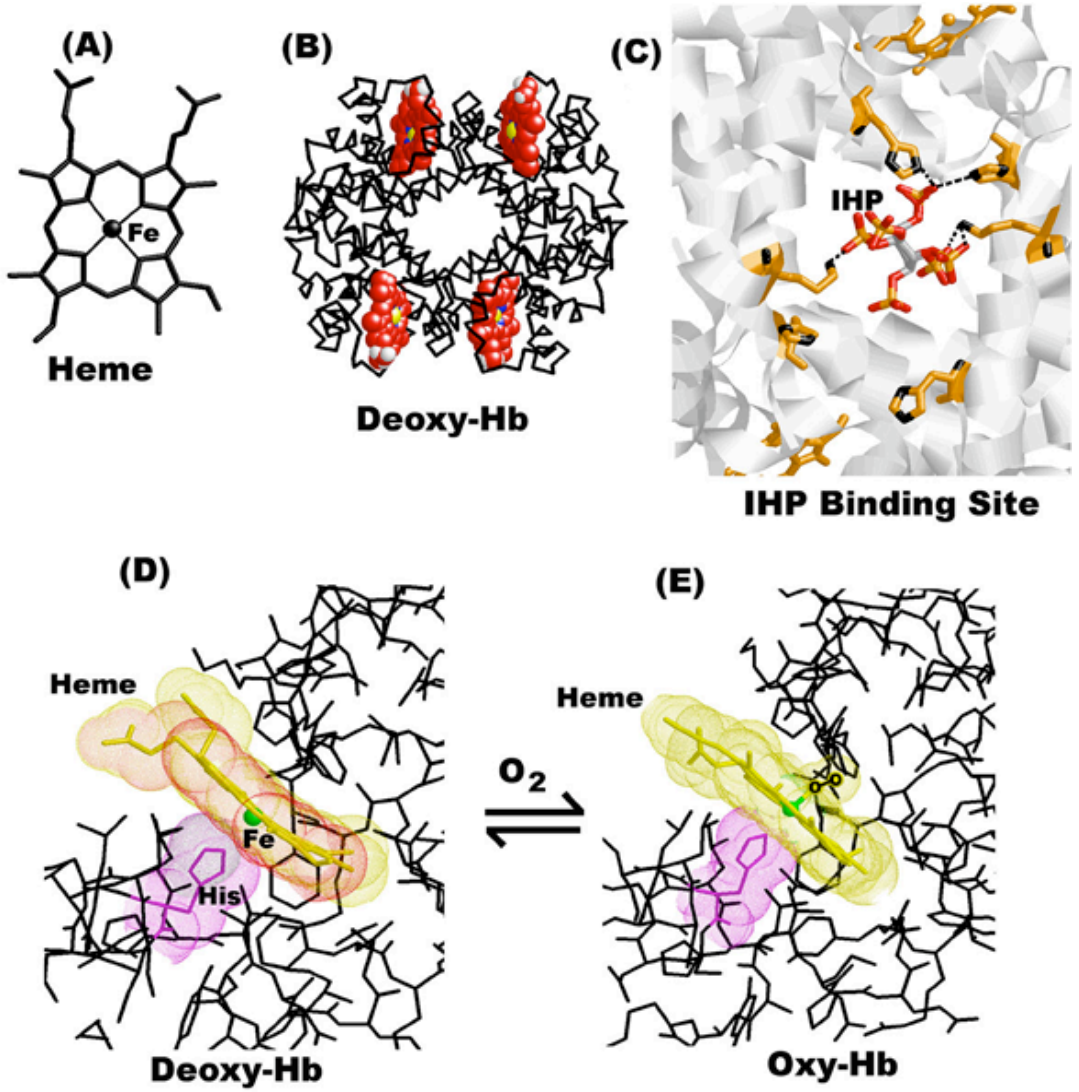
The secret of life

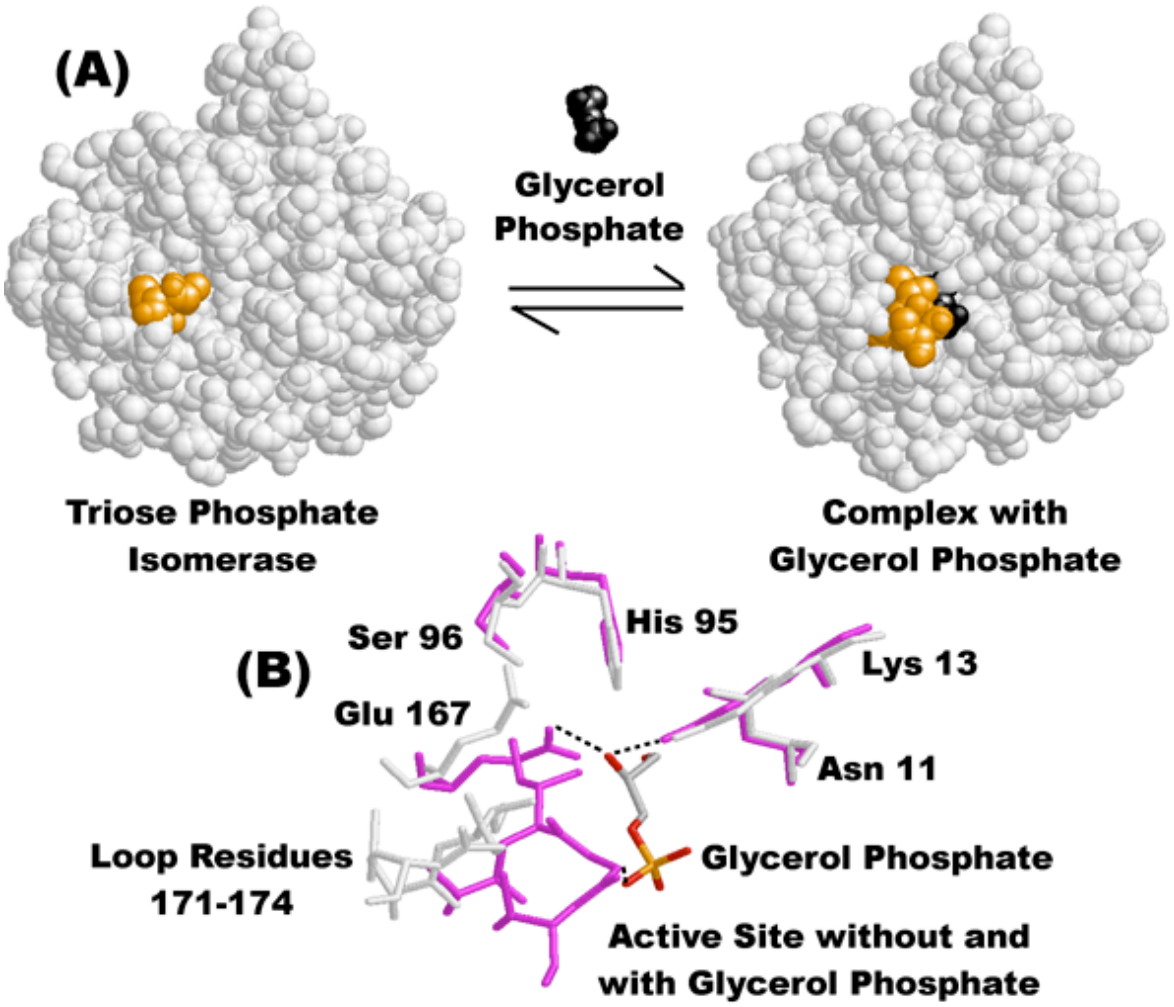
“The secret of life **is molecular recognition**; the ability of one molecule to "recognize" another through weak bonding interactions.”

Linus Pauling at the 25th anniversary of the Institute of Molecular Biology at the University of Oregon

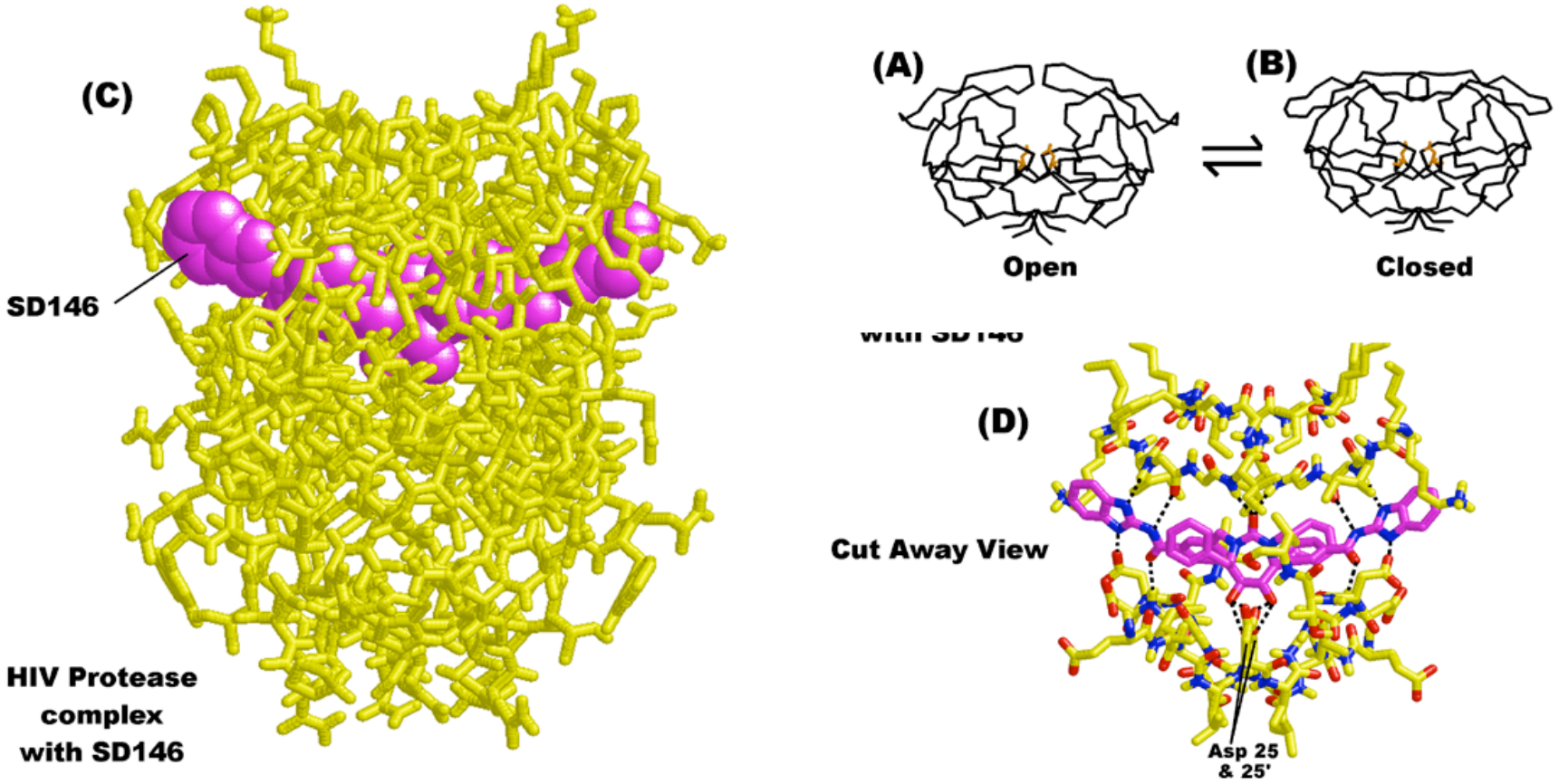
Binding of dioxygen to hemoglobin (air)



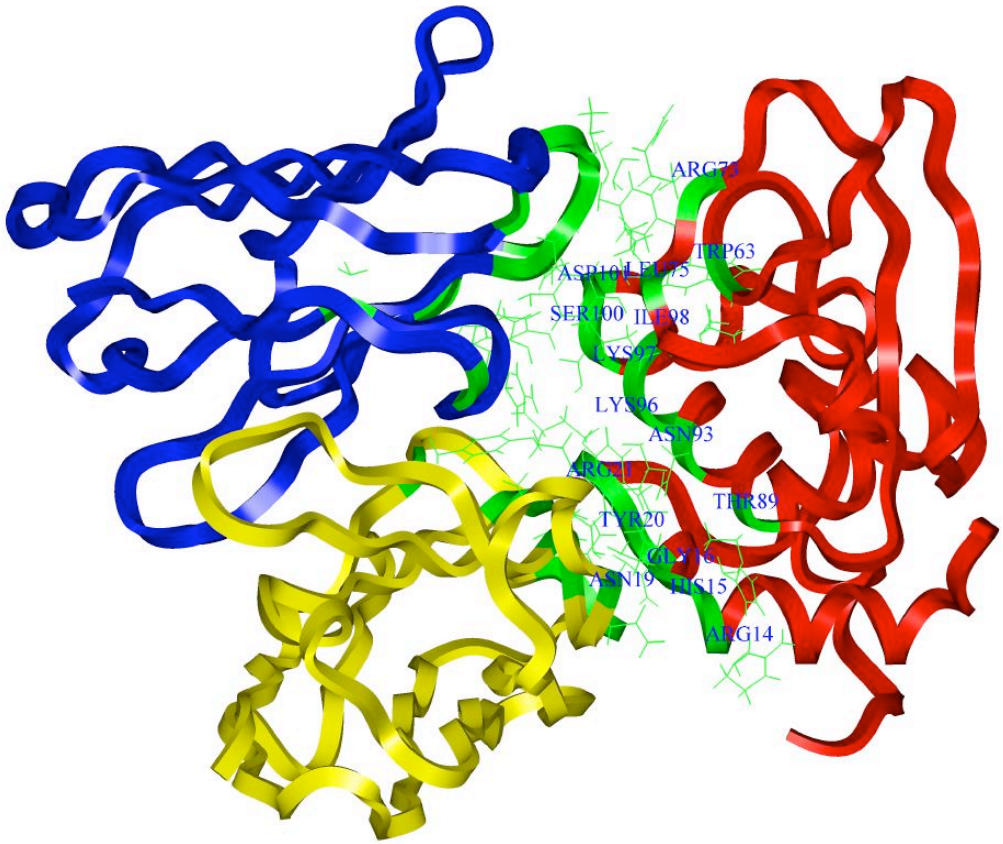
Binding of Glycerol-Phosphate to triose phosphate isomerase (energy)



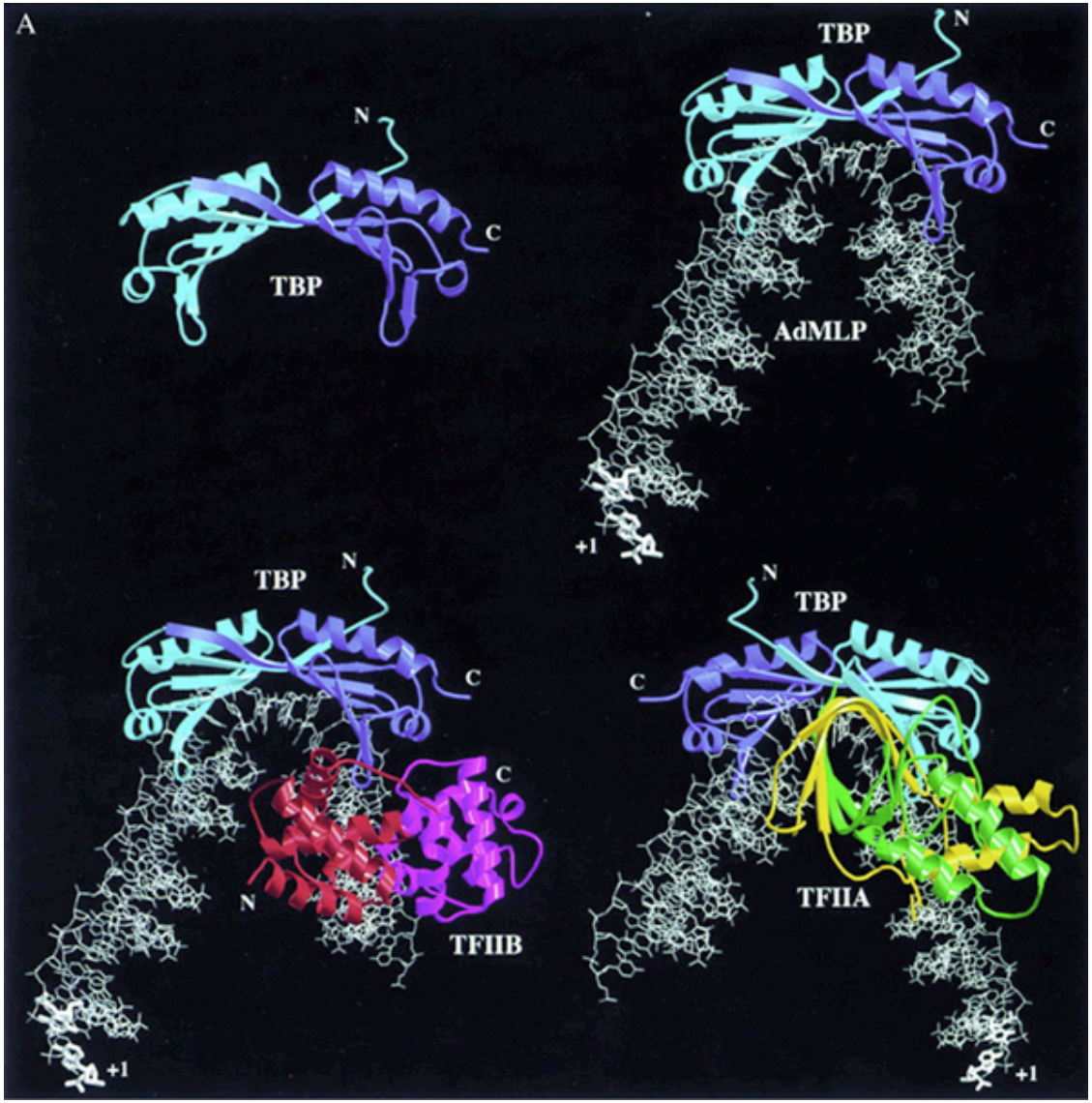
Complex of the HIV protease the inhibitor SD146 (drugs)



Antibody HyHEL-10 in complex with Hen Egg White Lyzoyme (immune system)

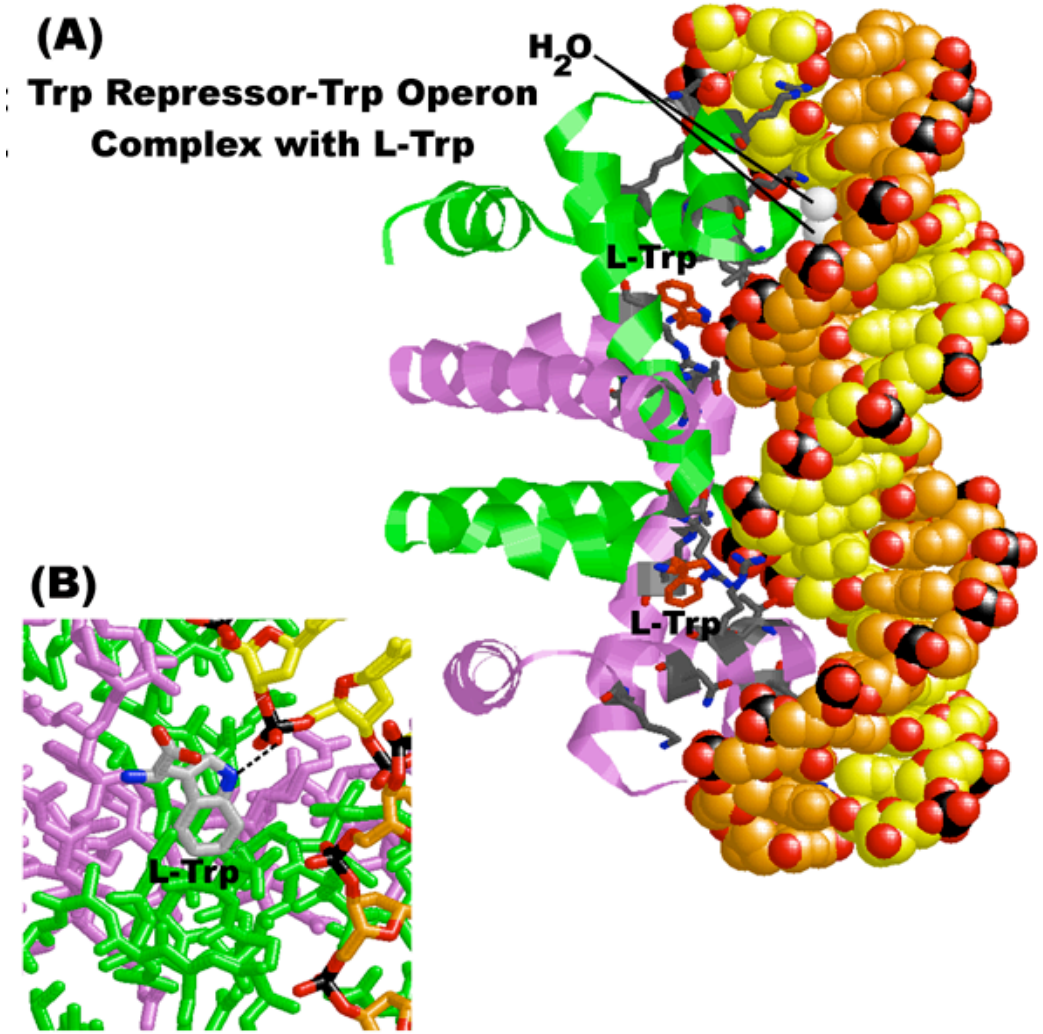


X-ray crystal structure of the TBP-promoter DNA complex (transcription starts here...)



Nikolov, D. B. & Burley, S. K. (1997). RNA polymerase II transcription initiation: a structural view. *PNAS* **94**, 15-22.

Structure of the tryptophan repressor with DNA (transcription regulation)



Binding of ligands to a macromolecule

- **General description of ligand binding**
  - the esssentials
  - thermodynamics
  - Adair equation

- **Simple equilibrium binding**
  - stoichiometric titration
  - equilibrium binding/dissociation constant

- **Complex equilibrium binding**
  - cooperativity
  - Scatchard plot and Hill Plot
  - MWC and KNF model for cooperative binding

The mass equation law for binding of a protein P to its DNA D

$$D_{\text{free}} + P_{\text{free}} \rightleftharpoons DP \qquad K_1 = \frac{D_{\text{free}} \cdot P_{\text{free}}}{DP}$$

binding of the first proteins with the dissociation constant K_1

D_{free}, concentration free DNA; P_{free}, concentration free protein

$$\text{binding constant } K_{\text{B}} = \frac{1}{\text{dissociation constant } K_{\text{D}}}$$

What is the meaning of the dissociation constant for binding of a single ligand to its site?

1. K_D is a concentration and has units of mol per liter

2. K_D gives the concentration of ligand that saturates 50% of the sites (when the total sit concentration ismuch lower than K_D)

3. Almost all binding sites are saturated if the ligand concentration is 10 x K_D

4. The dissociatin constant K_D is related to Gibbs free energy ΔG by the relation $\Delta G = - R\,T \ln(K_D)$

K_D values in biological systems

Movovalent ions binding to proteins or DNA have K_D 0.1 mM to 10 mM

Allosteric activators of enzymes e. g. NAD have K_D 0.1 μM to 0.1 mM

Site specific binding to DNA K_D 1 nM to 1 pM

Trypsin inhibitor to pancreatic trypsin protease K_D 0.01 pM

Antibody-antigen interaction have K_D 0.1 mM to 0.0001 pM

What is ΔG? The thermodynamics of a system

- **Biological systems can be usually described as having constant pressure *P* and constant temperature *T***
  - the system is free to exchange heat with the surrounding to remain at a constant temperature
  - it can expand or contract in volume to remain at atmospheric pressure

Some fundamentals of solution thermodynamics

- **At constant pressure *P* and constant temperature *T* the system is described by the Gibbs free energy:**

$$G \equiv H - TS \qquad \Delta G = \Delta H - T\Delta S$$

- ***H* is the enthalpy or heat content of the system, *S* is the entropy of the system**
- **a reaction occurs spontaneously only if $\Delta G < 0$**
- **at equilibrium $\Delta G = 0$**
- **for $\Delta G > 0$ the input of energy is required to drive the reaction**

the problem

In general we can **not assume** that the total free energy G of a solution consisting of *N* different components is simply the sum of the free energys of the single components.

The chemical potential μ of a substance is the partial molar Gibbs free energy

$$\overline{G_i} = \frac{\partial G}{\partial n_i} = \mu_i \qquad\qquad G = \sum_{i=1}^{n} n_i \cdot \mu_i$$

for an ideal solution it is: $\mu_i = \mu_i^0 + RT \ln C_i$

C_i is the concentration in mol per liter

μ_i^0 is the chemical potential of a substance at 1 mol/l

$$\mu_i = \mu_i^0 \quad for\ C_i = 1\ \text{mol/l}$$

Changes of the Gibbs free energy ΔG of an reaction

$$a\mathrm{A} + b\mathrm{B} + \ldots \rightleftharpoons g\mathrm{G} + h\mathrm{H} \ldots$$

$$\Delta G = G(\text{final state}) - G(\text{initial state})$$

$$\Delta G = g\,\mu_{\mathrm{G}} + h\,\mu_{\mathrm{H}} + \ldots - a\,\mu_{\mathrm{A}} - b\,\mu_{\mathrm{B}} - \ldots$$

from $\mu_{\mathrm{i}} = \mu_{\mathrm{i}}^{0} + RT \ln C_{\mathrm{i}}$ it follows:

$$\Delta G = g\,\mu_{\mathrm{G}}^{0} + h\,\mu_{\mathrm{H}}^{0} + \ldots - a\,\mu_{\mathrm{A}}^{0} - b\,\mu_{\mathrm{B}}^{0} - \ldots + RT \ln \frac{[\mathrm{G}]^{\mathrm{g}}[\mathrm{H}]^{\mathrm{h}} \ldots}{[\mathrm{A}]^{\mathrm{a}}[\mathrm{B}]^{\mathrm{b}} \ldots}$$

$$\Delta G = \Delta G^{0} + RT \ln \frac{[\mathrm{G}]^{\mathrm{g}}[\mathrm{H}]^{\mathrm{h}} \ldots}{[\mathrm{A}]^{\mathrm{a}}[\mathrm{B}]^{\mathrm{b}} \ldots}$$

ΔG of an reaction in equilibrium

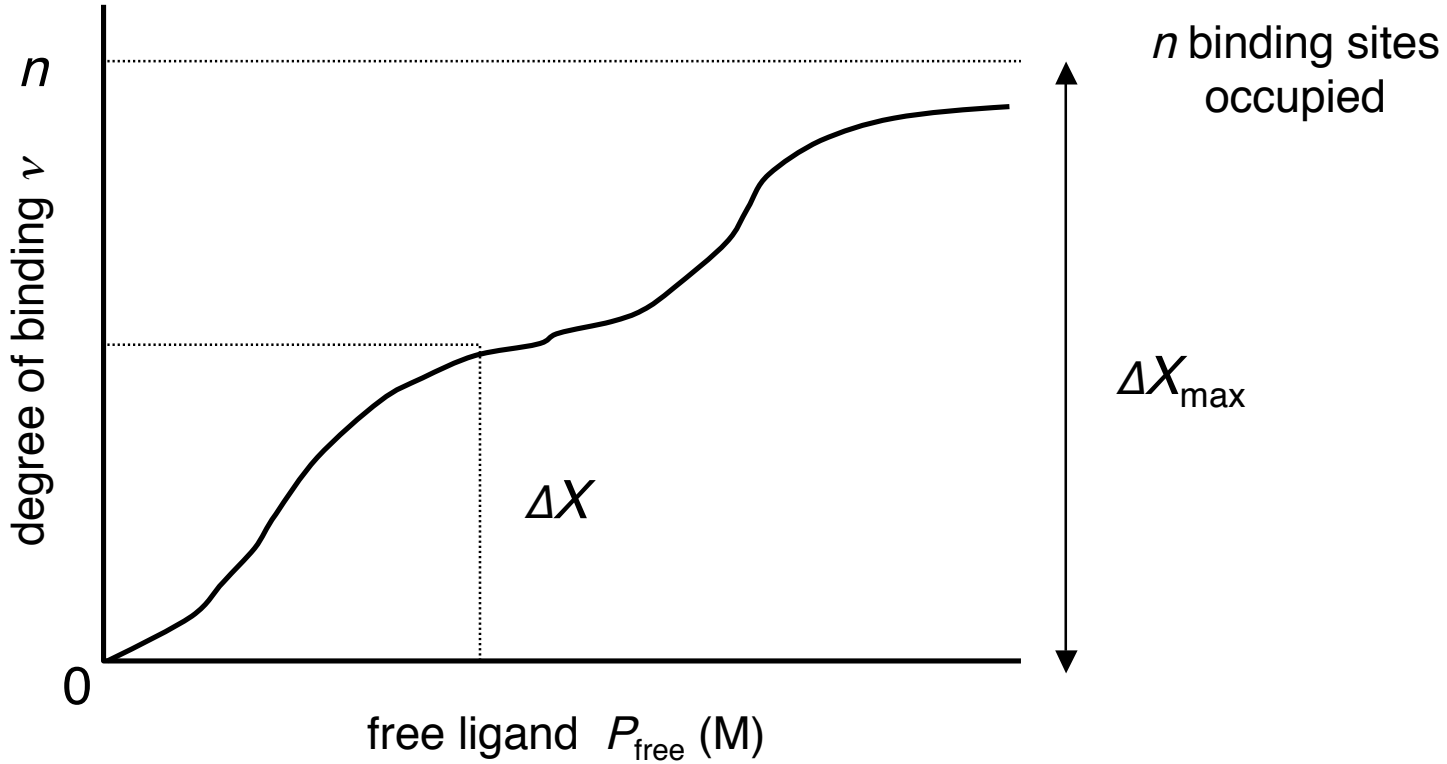
$$a\mathrm{A} + b\mathrm{B} + \ldots \rightleftharpoons g\mathrm{G} + h\mathrm{H} \ldots$$

$$0 = \Delta G^0 + RT \ln\left(\frac{[\mathrm{G}]^g[\mathrm{H}]^h \ldots}{[\mathrm{A}]^a[\mathrm{B}]^b \ldots}\right)_{\mathrm{Eq}}$$

$$\Delta G^0 = -RT \ln\left(\frac{[\mathrm{G}]^g[\mathrm{H}]^h \ldots}{[\mathrm{A}]^a[\mathrm{B}]^b \ldots}\right)_{\mathrm{Eq}} = -RT \ln K$$

$$K = \left(\frac{[\mathrm{G}]^g[\mathrm{H}]^h \ldots}{[\mathrm{A}]^a[\mathrm{B}]^b \ldots}\right)_{\mathrm{Eq}} = \exp\left(\frac{-\Delta G^0}{RT}\right)$$

Titration of a macromolecule *D* with *n* binding sites for the ligand *P* which is added to the solution



$$\frac{\Delta X}{\Delta X_{\max}} = \frac{\nu}{n} = \theta \text{ (fraction saturation)} \qquad \nu = \frac{[\text{bound ligand } P]}{[\text{macromolecule } D]}$$

Schematic view of gel electrophoresis to analyze protein-DNA complexes

Mark M. Garner and Arnold Revzin

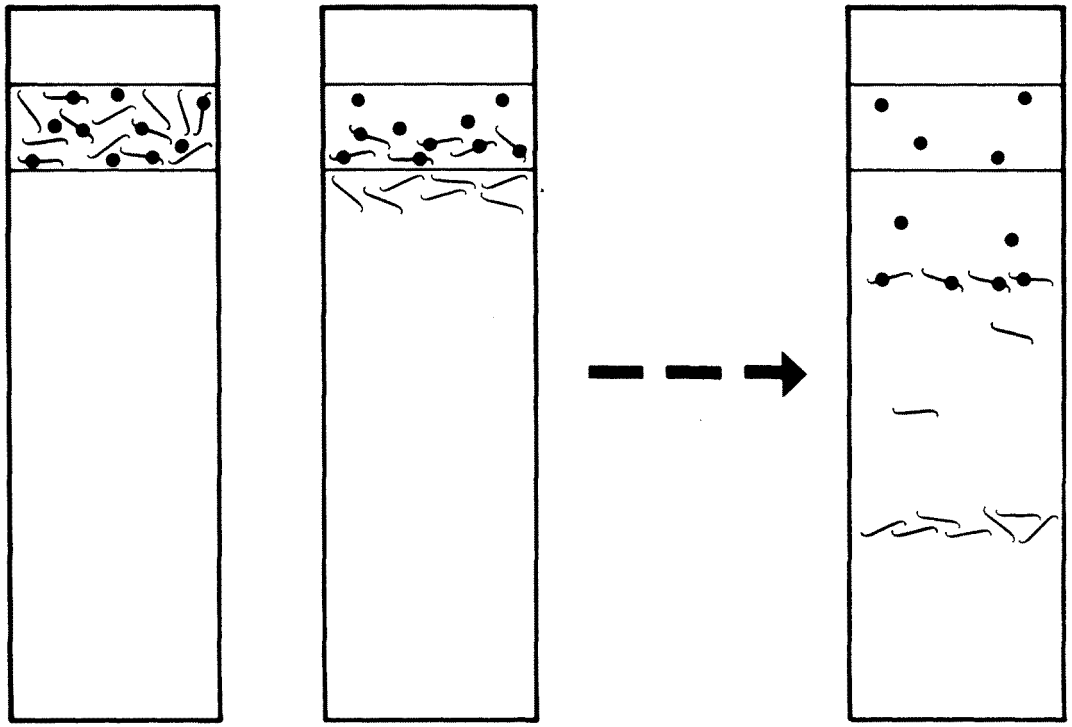
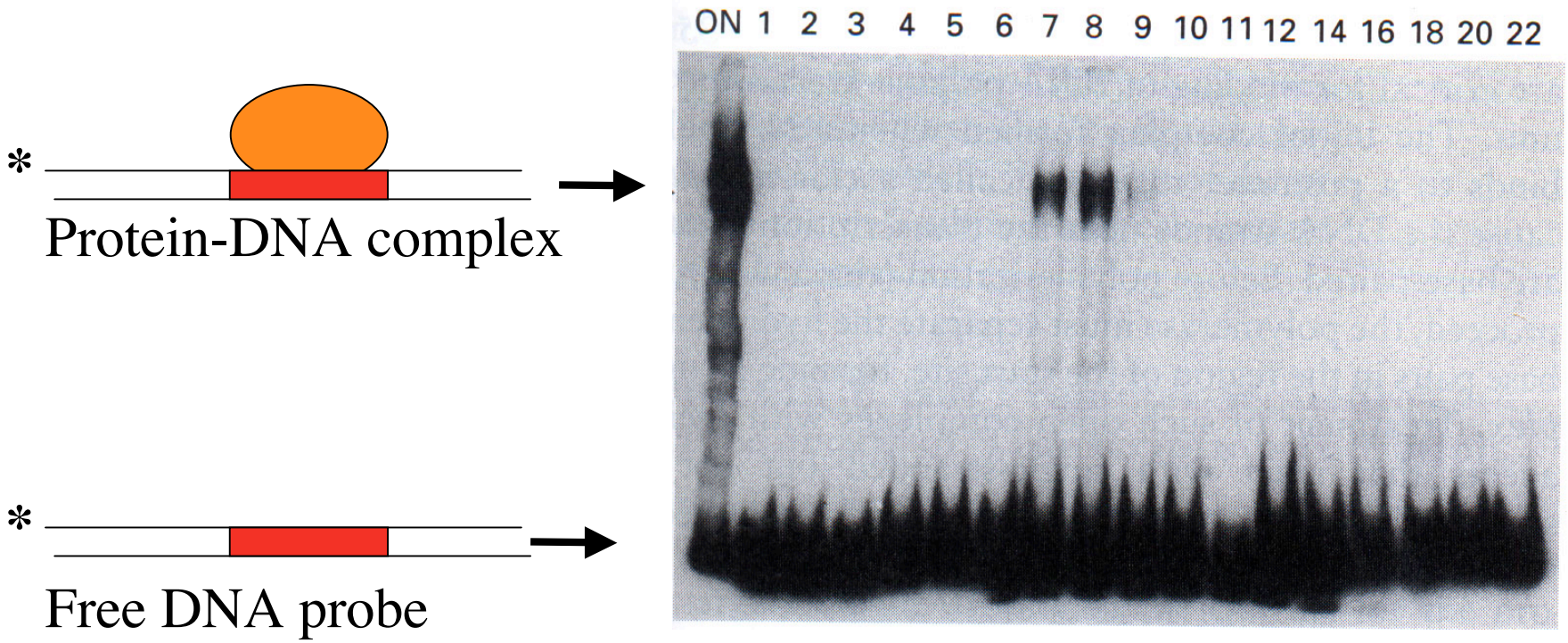


Figure 1. Schematic diagram of the gel retardation method. The filled circles represent protein, curved lines represent linear DNA fragments. The left-hand panel illustrates the DNA – protein solution loaded on to the gel. The middle panel shows free DNA entering the gel just after the power is turned on. The right-hand panel depicts the situation later in the run; bands of complexes and of free DNA are seen. If the complexes dissociate during electrophoresis, the DNA released never catches up with the main band of free DNA.

“Gel shift”: electorphoretic mobility shift assay (“EMSA”) for DNA-binding proteins



1. Prepare labeled DNA probe
2. Bind protein
3. Native gel electrophoresis

Advantage: sensitive, fmol DNA

Disadvantage: requires stable complex; little “structural” information about which protein is binding

EMSA of Lac repressor binding to operator DNA

From (a) to (j) the concentration
of lac repressor is increased.

Complexes with

Free DNA

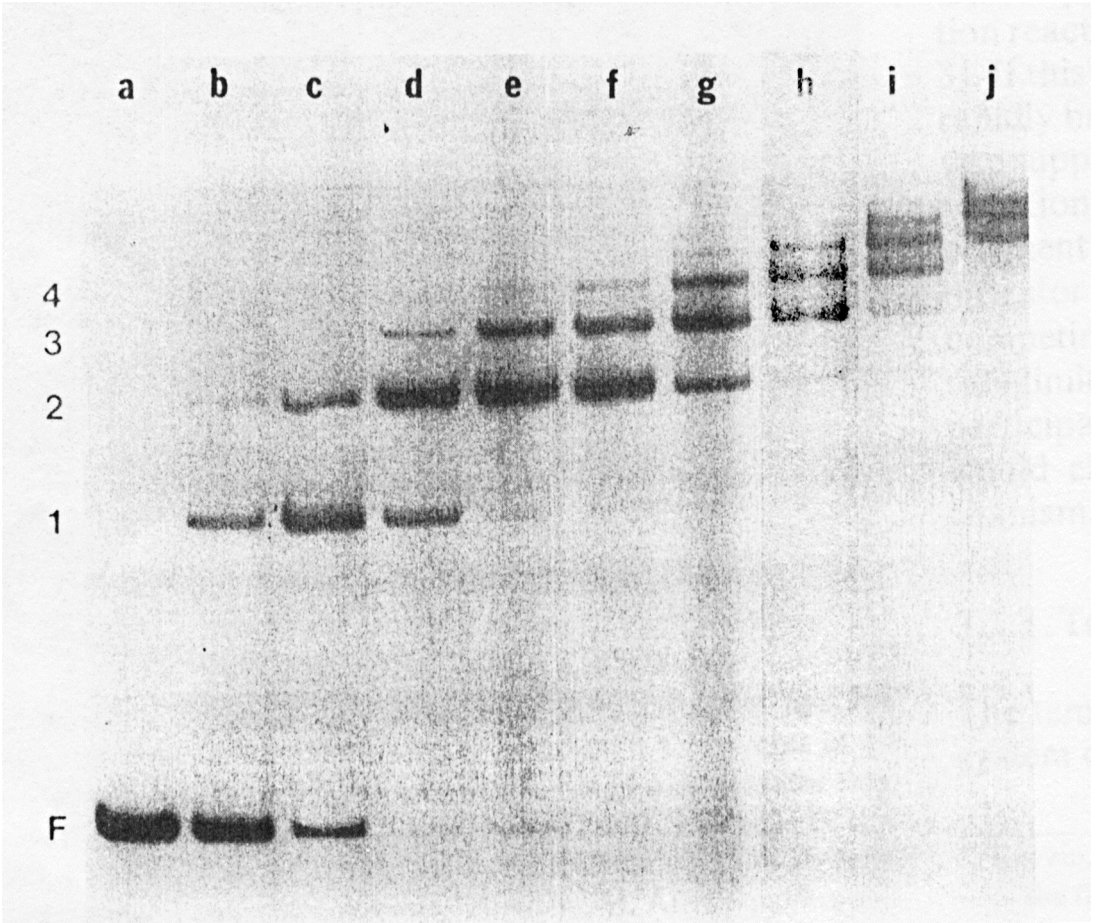
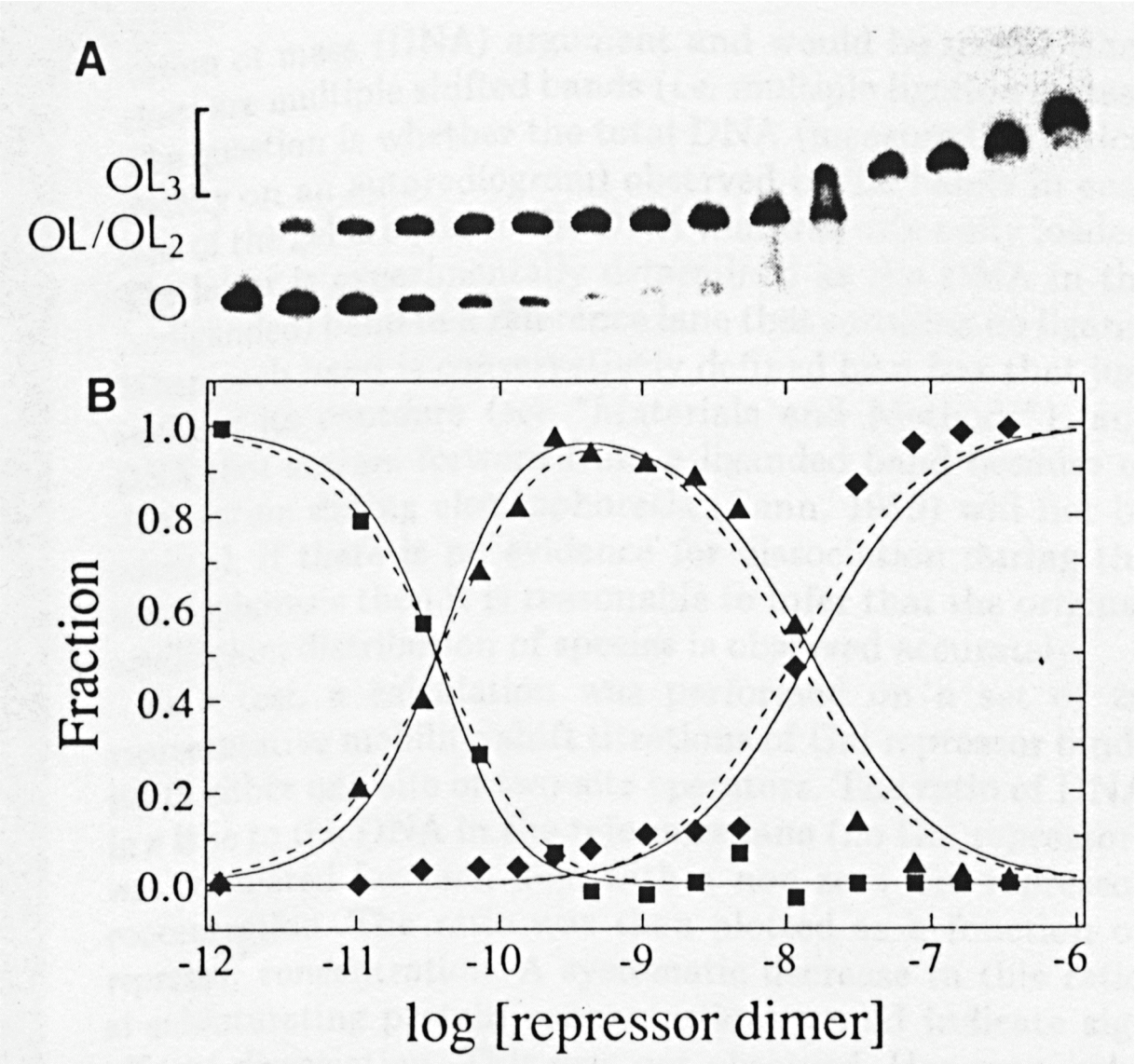
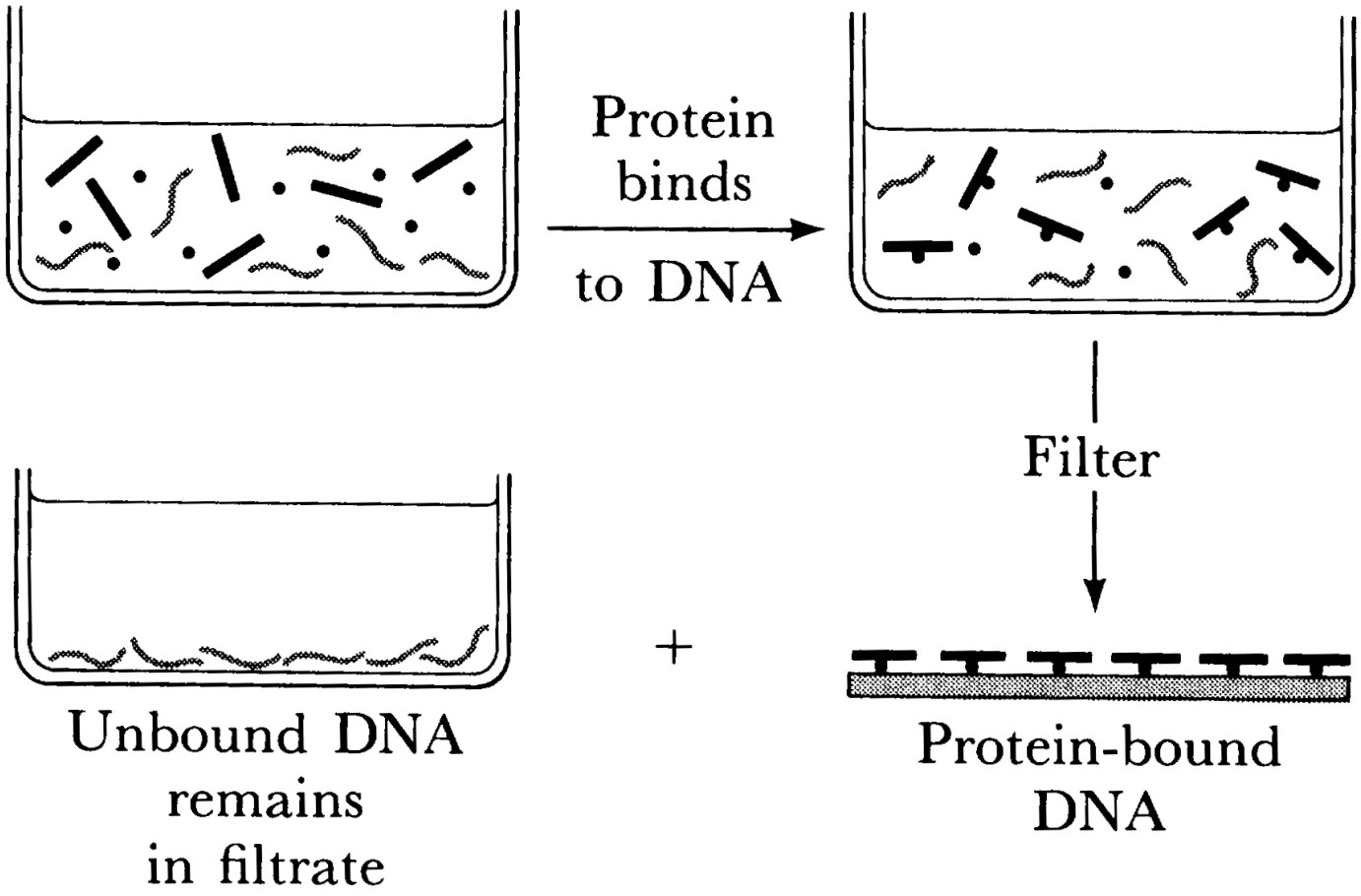


Figure 1. Titration of the 203 bp L8-UV5 lactose promoter-operator fragment with *lac* repressor. The DNA fragment concentration was 18.5 nM *Lac* repressor concentrations were: 0, 12.4, 24.8, 37.0, 49.4, 61.6, 74.0, 98.8, 123.4 and 148.0 nM for samples (a)–(j) respectively. Samples were incubated for 30 min at room temperature in 10 mM Tris (pH 8.0 at 21 °C), 1 mM EDTA, 50 mM KCl, and applied to a 5 % polyacrylamide gel equilibrated with the same buffer. Electrophoresis was at 8 V/cm for 2 h. The repressor: fragment ratios [1] of some complexes are given of the left margin. Band F denotes free DNA.

Measuring binding constants for lambda repressor on a gel

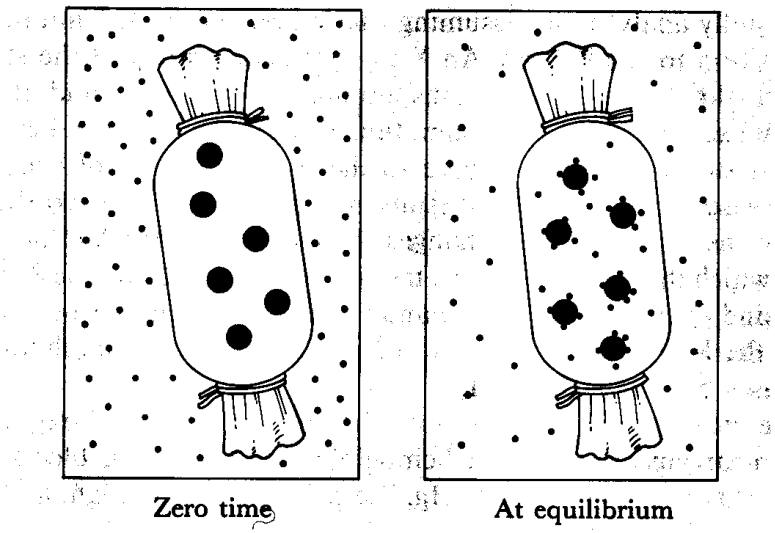


Principle of filter-binding assay



Binding measurments by equilibrium dialysis

A macromolecule is dialyzed against a solution of ligand. Upon reaching equilibrium, the ligand concentration is measured inside and outside the dialysis chamber. The excess ligand inside the chamber corresponds to bound ligand.



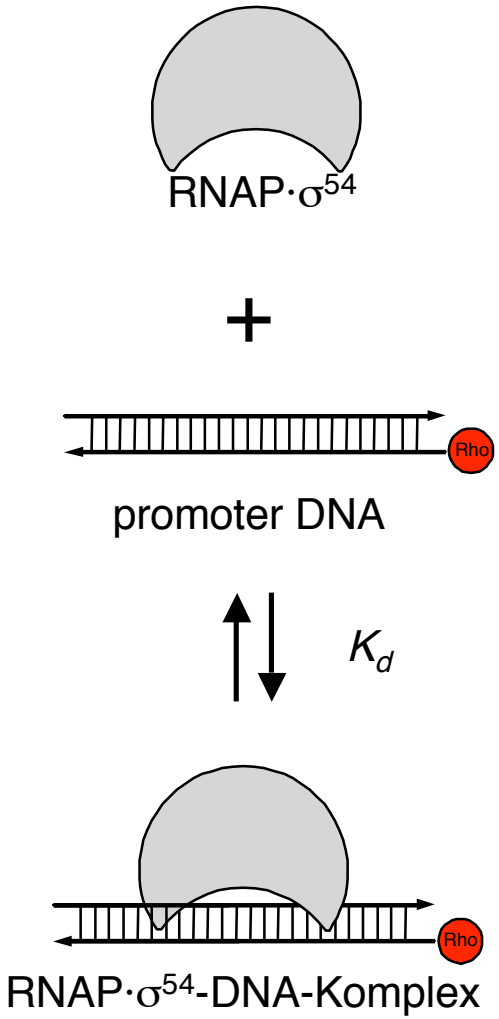
$$\nu = \frac{[X]_{in} - [X]_{out}}{M}$$

- direct measurement of binding

-non-specific binding will obscure results, work at moderate ionic strength (≥ 50 to avoid the *Donnan Effect* (electrostatic interactions between the macromolecule and a charged ligand.

- needs relatively large amounts of material

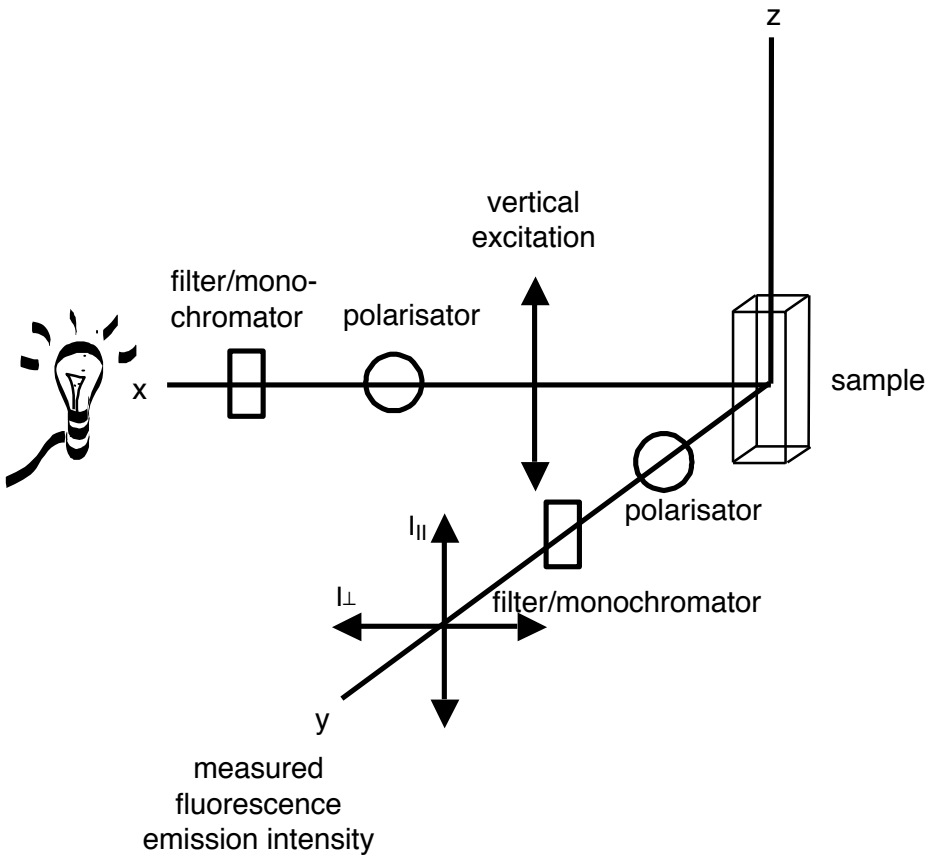
Analysis of binding of RNAP·σ^{54} to a promoter DNA sequence by measurements of fluorescence anisotropy



free DNA with a fluorophore
with high rotational diffusion
-> low fluorescence anisotropy r_{min}

RNAP-DNA complex
with low rotational diffusion
-> high fluorescence anisotropy
r_{max}

How to measure binding of a protein to DNA?
One possibility is to use fluorescence anisotropy

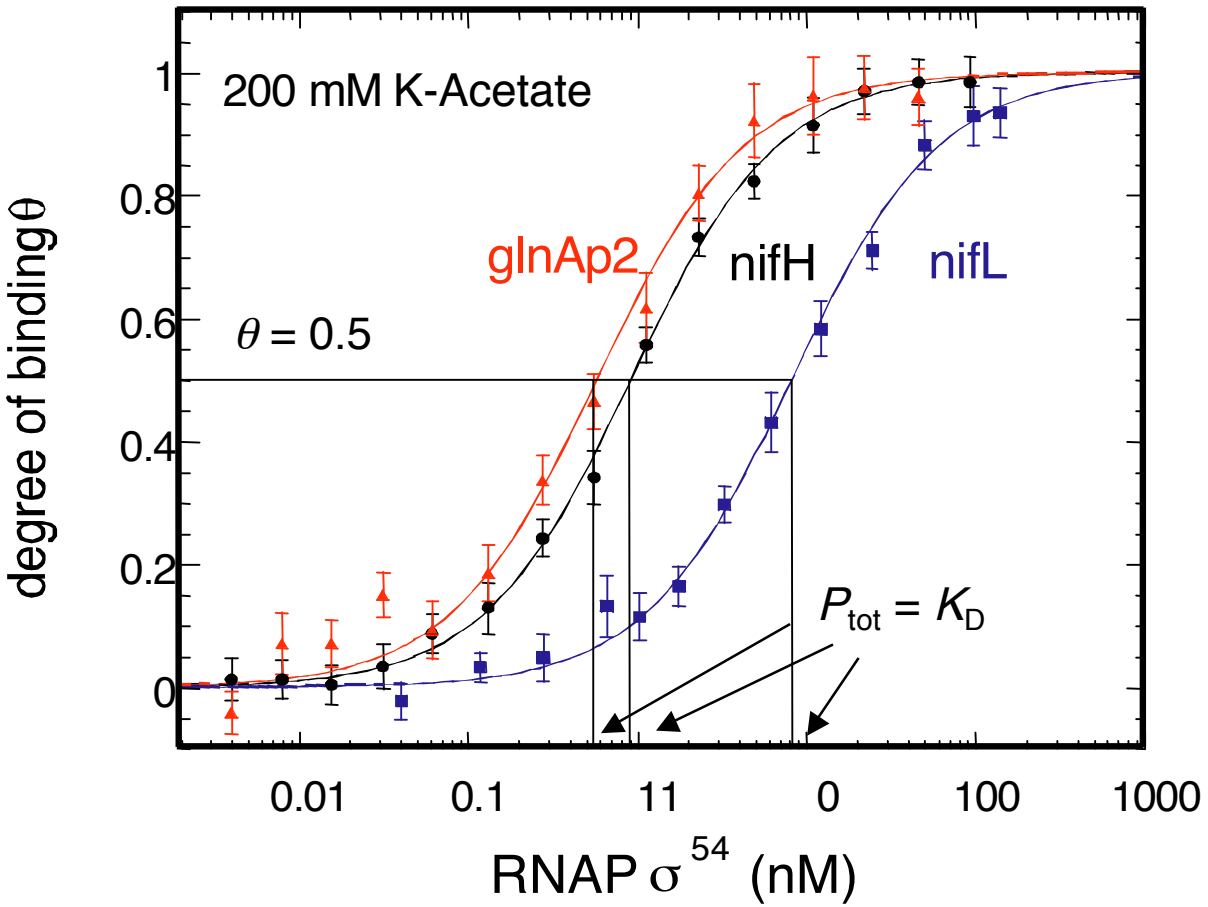


$$r = \frac{I_{II} - I_{\perp}}{I_{II} + 2I_{\perp}}$$

Definition of fluorescence anisotropy *r*

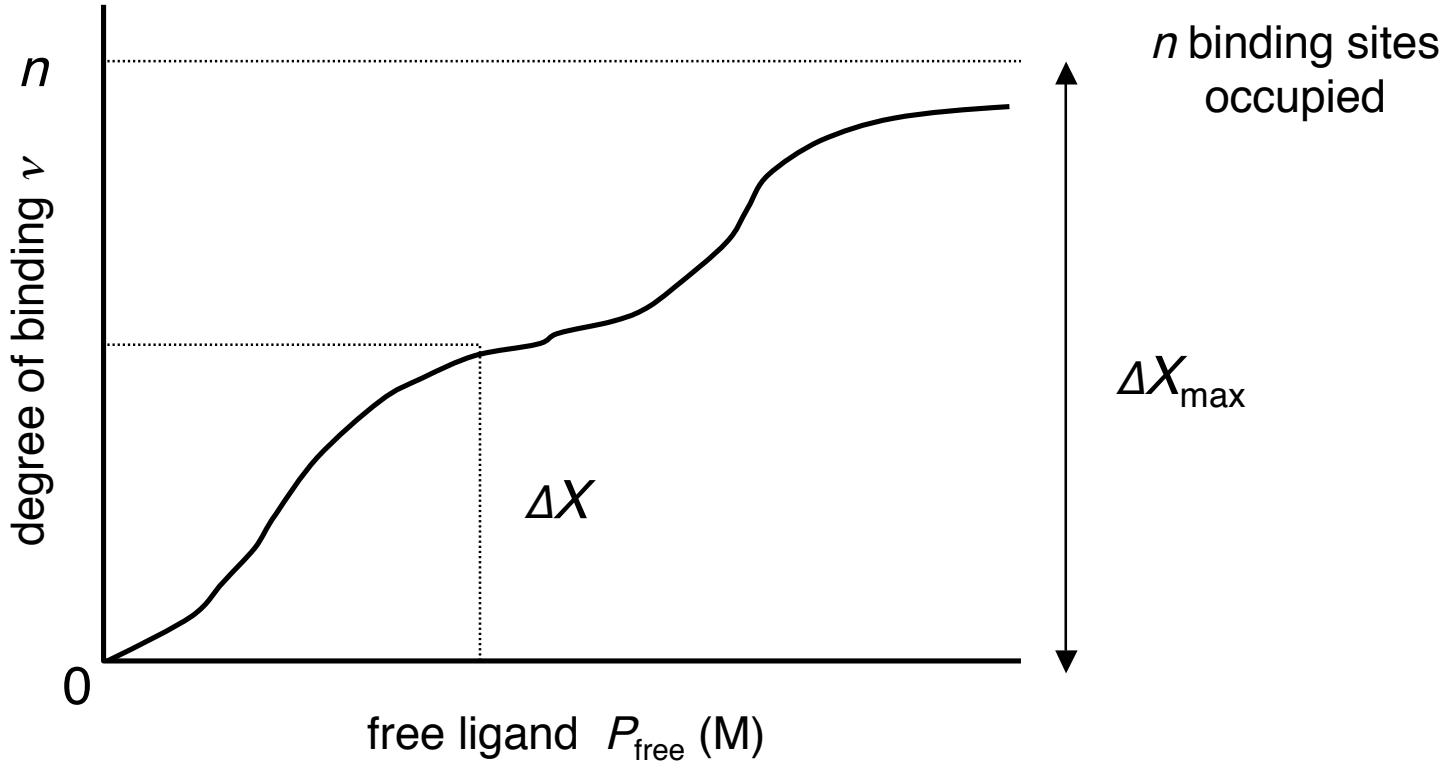
The anisotropy *r* reflects the rotational diffusion of a fluorescent species

Measurements of fluorescence anisotropy to monitor binding of RNAP·σ^{54} to different promoters



Vogel, S., Schulz A. & Rippe, K.

Titration of a macromolecule *D* with *n* binding sites for the ligand *P* which is added to the solution



$$\frac{\Delta X}{\Delta X_{\max}} = \frac{\nu}{n} = \theta \text{ (fraction saturation)} \qquad \nu = \frac{[\text{bound ligand } P]}{[\text{macromolecule } D]}$$

Example: binding of a protein *P* to a DNA-fragment *D* with one or two binding sites

$$D_{\text{free}} + P_{\text{free}} \rightleftharpoons DP \qquad K_1 = \frac{D_{\text{free}} \cdot P_{\text{free}}}{DP}$$

binding of the first proteins with the dissociation constant K_1

D_{free}, concentration free DNA; P_{free}, concentration free protein;
DP, complex with one protein; DP_2, complex with two proteins;

$$DP + P_{\text{free}} \rightleftharpoons DP_2 \qquad K_2 = \frac{DP \cdot P_{\text{free}}}{DP_2}$$

binding of the second proteins with the dissociation constant K_2

$$D + 2P_{\text{free}} \rightleftharpoons DP_2 \qquad K_2^* = \frac{D_{\text{free}} \cdot P_{\text{free}}^2}{DP_2} \qquad K_2^* = K_1 \cdot K_2$$

alternative expression

$$\text{binding constant } K_B = \frac{1}{\text{dissociation constant } K_D}$$

Definition of the degree of binding ν

$$\nu = \frac{[\text{bound ligand } P]}{[\text{macromolecule } D]}$$

degree of binding ν

$$\nu_1 = \frac{DP}{D_{\text{free}} + DP}$$

ν for one binding site

$$\nu_2 = \frac{DP + 2 \cdot DP_2}{D_{\text{free}} + DP + DP_2}$$

ν for two binding sites

$$\nu = \frac{\sum_{i=1}^{n} i \cdot \frac{1}{K_i} \cdot D_{\text{frei}} \cdot P_{\text{frei}}^{i}}{\sum_{i=0}^{n} \frac{1}{K_i} \cdot D_{\text{frei}} \cdot P_{\text{frei}}^{i}} = \frac{\sum_{i=1}^{n} i \cdot \frac{1}{K_i} \cdot P_{\text{frei}}^{i}}{\sum_{i=0}^{n} \frac{1}{K_i} \cdot P_{\text{frei}}^{i}} \qquad \text{mit } K_0 = 1$$

ν for *n* binding sites (Adair equation)

Binding to a single binding site: Deriving an expression for the degree of binding ν or the fraction saturation θ

$$D_{\text{free}} + P_{\text{free}} \rightleftharpoons DP \qquad K_{\text{D}} = \frac{D_{\text{free}} \cdot P_{\text{free}}}{DP}$$

from the Adair equation we obtain:

$$\nu_1 = \theta = \frac{\frac{1}{K_{\text{D}}} \cdot P_{\text{free}}}{1 + \frac{1}{K_{\text{D}}} \cdot P_{\text{free}}} \quad \Leftrightarrow \quad \nu_1 = \theta = \frac{P_{\text{free}}}{K_{\text{D}} + P_{\text{free}}}$$

Often the concentration P_{free} can not be determined but the total concentration of added protein P_{tot} is known.

$$P_{\text{free}} = P_{\text{tot}} - \nu_1 \cdot D_{\text{tot}}$$

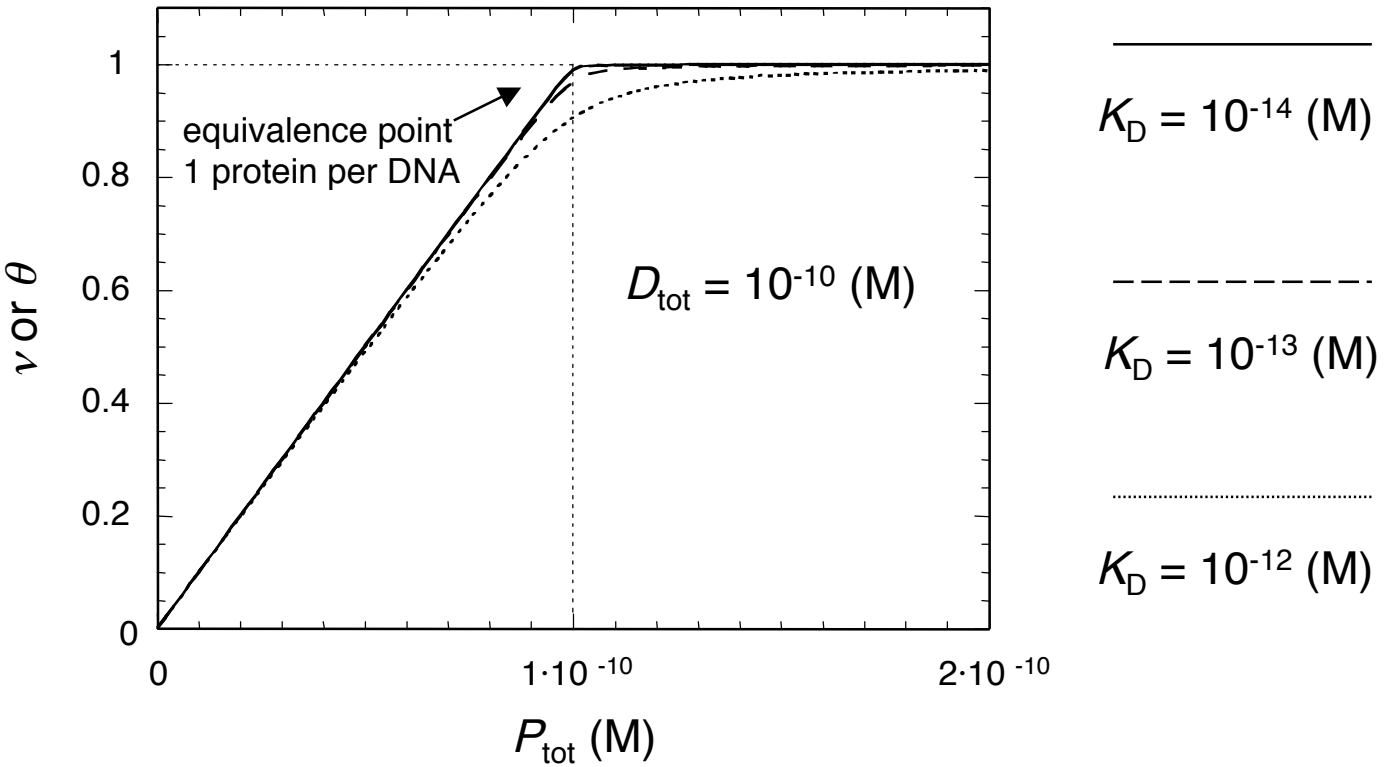
$$\nu_1 = \frac{D_{\text{tot}} + P_{\text{tot}} + K_{\text{D}} - \sqrt{\left(D_{\text{tot}} + P_{\text{tot}} + K_{\text{D}}\right)^2 - 4 \cdot D_{\text{tot}} \cdot P_{\text{tot}}}}{2 \cdot D_{\text{tot}}}$$

Stoichiometric titration to determine the number of binding sites

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

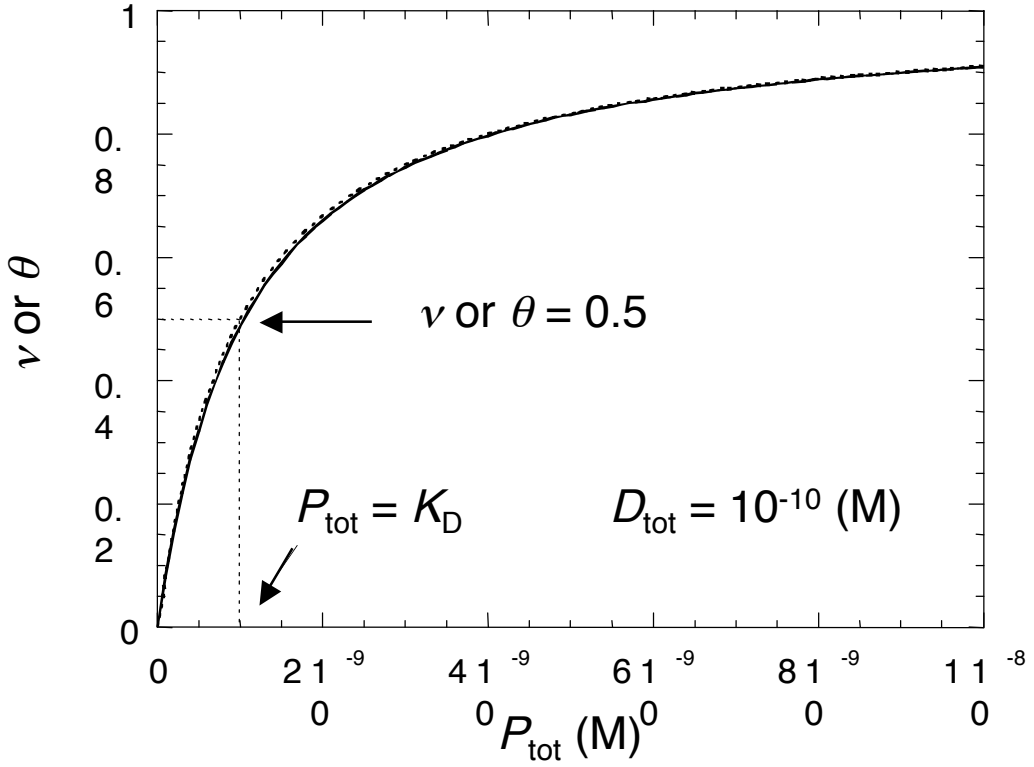
for $n = 1$

$$\nu = \theta$$



To a solution of DNA strands with a single binding site small amounts of protein *P* are added. Since the binding affinity of the protein is high (low K_D value as compared to the total DNA concentration) practically every protein binds as long as there are free binding sites on the DNA. This is termed “stoichiometric binding” or a “stoichiometric titration”.

Binding to a single binding site. Titration of DNA with a protein for the determination of the dissociation constant K_D

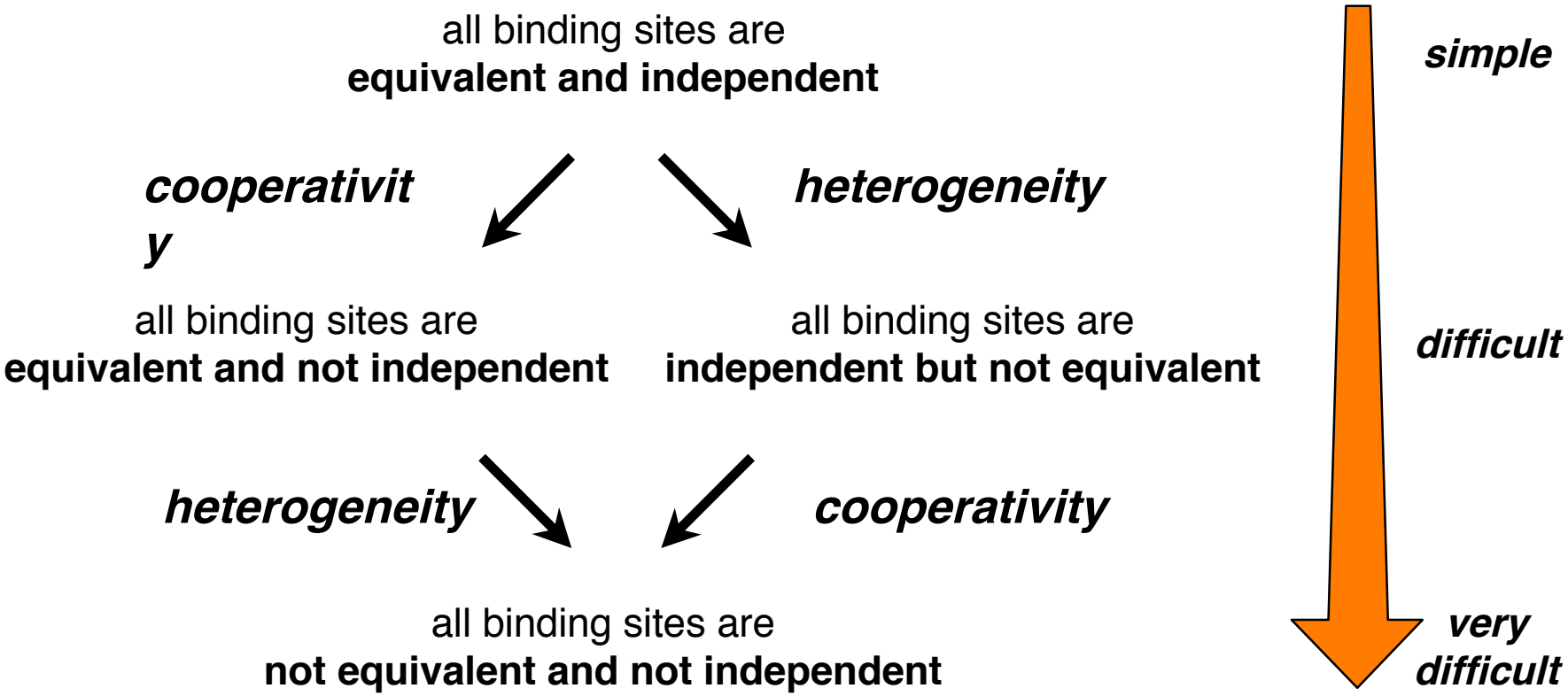


$K_D = 10^{-9}$ (M) $\quad \nu_1 = \dfrac{P_{free}}{P_{free} + K_D}$

$K_D = 10^{-9}$ (M) $\quad \nu_1 = \dfrac{P_{tot}}{P_{tot} + K_D}$

$$\nu_1 = \frac{P_{free}}{P_{free} + K_D} \approx \frac{P_{tot}}{P_{tot} + K_D} \quad \text{if } P_{free} \approx P_{tot} \quad \text{d. h. } 10 \times D_{tot} \leq K_D$$

Increasing complexity of binding



Binding to *n* identical binding sites

$$\nu_1 = \frac{P_{\text{free}}}{P_{\text{free}} + K_{\text{D}}}$$

binding to a single binding site

$$\nu_{\text{n}} = \frac{n \cdot P_{\text{free}}}{k_{\text{D}} + P_{\text{free}}}$$

binding to *n* independent and identical binding sites

$$D + n \cdot P_{\text{free}} \rightleftharpoons DP_{\text{n}} \qquad K_{\text{n}} = \frac{D_{\text{free}} \cdot P_{\text{free}}^{\text{n}}}{DP_{\text{n}}} \qquad \nu_{\text{n}} = \frac{n \cdot P_{\text{free}}^{\text{n}}}{K_{\text{n}} + P_{\text{free}}^{\text{n}}}$$

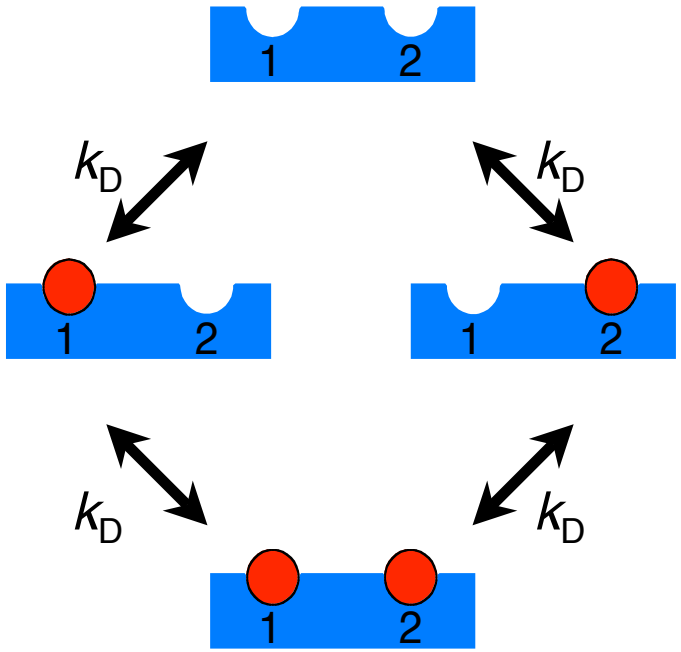
strong cooperative binding to *n* identical binding sites

$$\nu_{\text{n}} = \frac{n \cdot P_{\text{free}}^{\alpha_{\text{H}}}}{K^{\alpha_{\text{H}}} + P_{\text{free}}^{\alpha_{\text{H}}}}$$

approximation for cooperative binding to *n* identical binding sites, α_{H} Hill coefficient

Difference between microscopic and macroscopic dissociation constant

microscopic binding



macroscopic binding

D_{free}

2 possibilities for the formation of *DP*

DP

$$K_1 = \frac{k_D}{2}$$

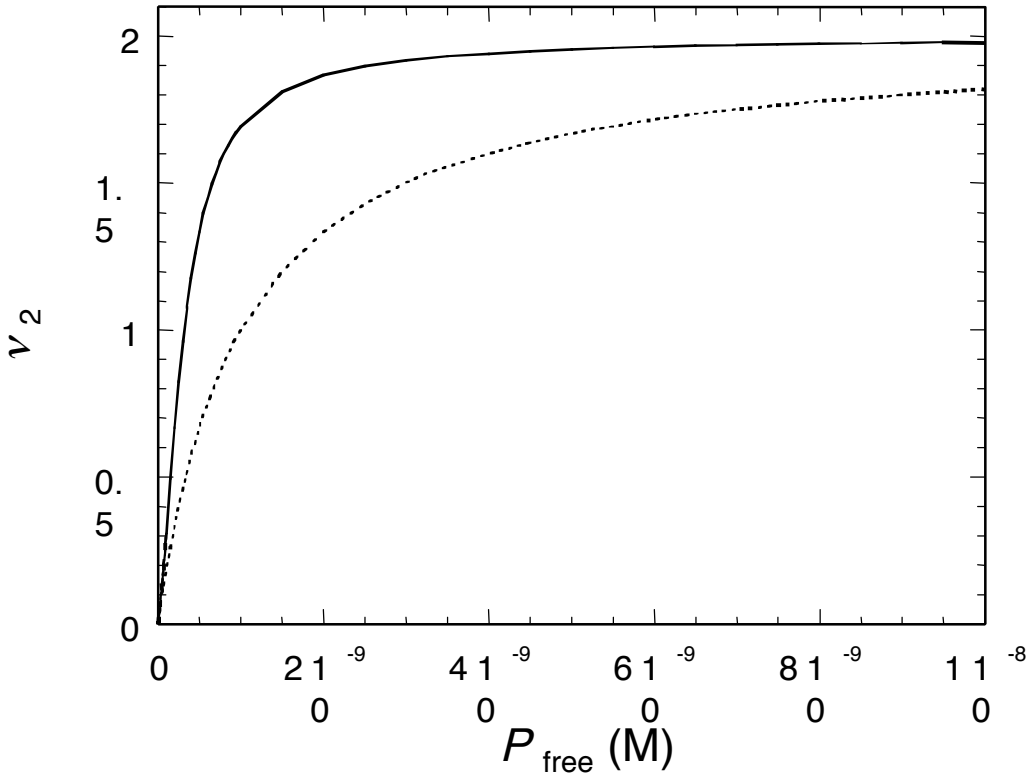
2 possibilities for the dissociation of DP_2

DP_2

$$K_2 = 2 \cdot k_D$$

$$\frac{K_1}{K_2} = \frac{k_D/2}{2 \cdot k_D} = \frac{1}{4}$$

Cooperativity: the binding of multiple ligands to a macromolecule is not independent



independent binding
microscopic binding constant
$k_D = 10^{-9}$ (M)

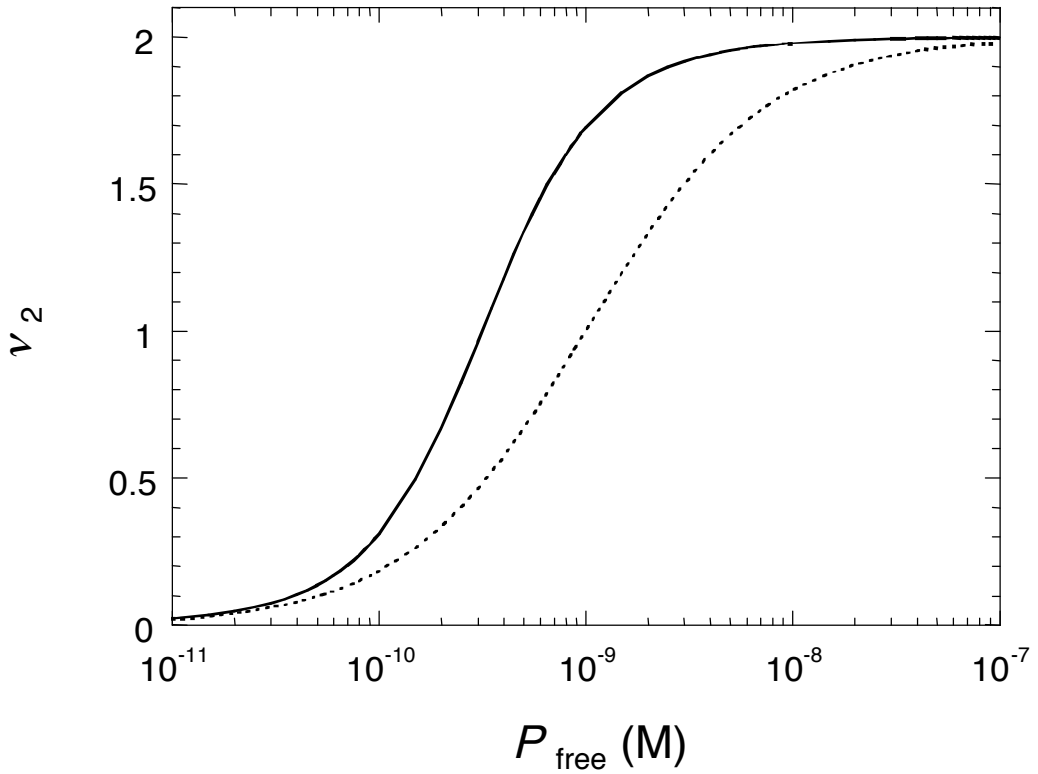
macroscopic binding constants
$K_1 = 5 \cdot 10^{-10}$ (M); $K_2 = 2 \cdot 10^{-9}$ (M)

cooperative binding
microscopic binding constant
$k_D = 10^{-9}$ (M)

macroscopic binding constants
$K_1 = 5 \cdot 10^{-10}$ (M); $K_2 = 2 \cdot 10^{-10}$ (M)

Adair equation: $$\nu_2 = \frac{K_2 \cdot P_{free} + 2 \cdot P_{free}^2}{K_1 \cdot K_2 + K_2 \cdot P_{free} + P_{free}^2}$$

Logarithmic representation of a binding curve



independent binding
microscopic binding constant
$k_D = 10^{-9}$ (M)

macroscopic binding constants
$K_1 = 5 \cdot 10^{-10}$ (M); $K_2 = 2 \cdot 10^{-9}$ (M)

cooperative binding
microscopic binding constant
$k_D = 10^{-9}$ (M)

macroscopic binding constants
$K_1 = 5 \cdot 10^{-10}$ (M); $K_2 = 2 \cdot 10^{-10}$ (M)

• Determine dissociation constants over a ligand concentration of at least three orders of magnitudes

• Logarithmic representation since the chemical potential μ is proportional to the logarithm of the concentration.

Scatchard Plot

The hyperbolic binding curve can be put in a linear form by plotting Y/[L] versus Y. Starting with equation (3):

$$Y = \frac{[L]K_{eq}}{1 + [L]K_{eq}}$$

Y = θ (degree of binding)
L: free ligand

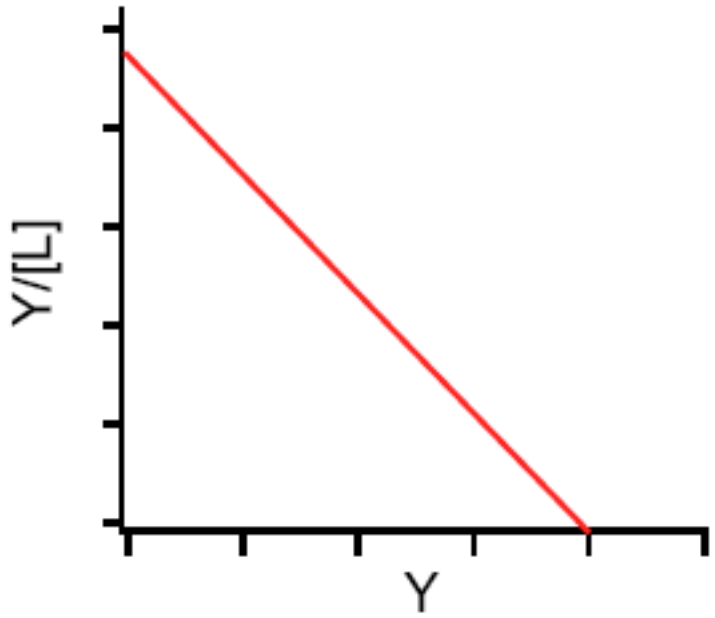
$$Y + Y[L]K_{eq} = [L]K_{eq}$$

divide by [L]

$$\frac{Y}{[L]} + YK_{eq} = K_{eq}$$

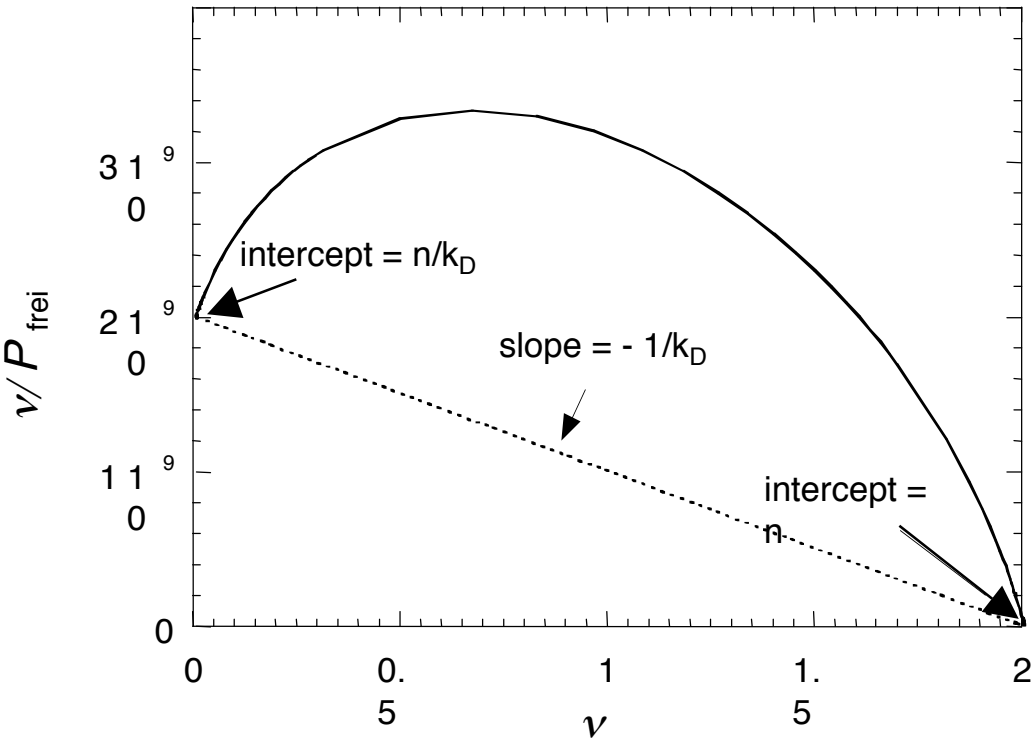
$$\frac{Y}{[L]} = K_{eq} - YK_{eq}$$

$$\frac{Y}{[L]} = \frac{1}{K_d} - \frac{Y}{K_d}$$



The slope of this plot is $-K_{eq}$ (or $-1/K_d$).
The y-intercept is $1/K_d$.
The x-intercept is the number of binding sites (B_{max} = stoichiometry).

Visualisation of binding data - Scatchard plot



$$\nu_n = \frac{n \cdot P_{free}}{k_D + P_{free}} \Leftrightarrow \frac{\nu_n}{P_{free}} = \frac{n}{k_D} - \frac{\nu_n}{k_D}$$

independent binding
microscopic binding constant
$k_D = 10^{-9}$ (M)

macroscopic binding constants
$K_1 = 5 \cdot 10^{-10}$ (M); $K_2 = 2 \cdot 10^{-9}$ (M)

cooperative binding
microscopic binding constant
$k_D = 10^{-9}$ (M)

macroscopic binding constants
$K_1 = 5 \cdot 10^{-10}$ (M); $K_2 = 2 \cdot 10^{-10}$ (M)

Problems with Scatchard Plots

In the life sciences, linearization often is used to simplify the analysis of quantitative data that could be analyzed more accurately by nonlinear regression programs using a computer.

Scientists have traditionally preferred linear regression methods such as the Scatchard plot to nonlinear regression methods because of their inherent simplicity.

However, linearization methods can generate systematic error and the K_d and B_{max} values you determine by linear regression of Scatchard transformed data are likely to be far from their true values.

After analyzing your data with nonlinear regression, however, it is often useful to **display** data as a Scatchard plot. Scatchard plots are often shown as insets to the saturation binding curves. They are especially useful when you want to show a change in B_{max} or K_d.

Binding to *n* identical binding sites

$$\nu_1 = \frac{P_{\text{free}}}{P_{\text{free}} + K_{\text{D}}}$$

binding to a single binding site

$$\nu_{\text{n}} = \frac{n \cdot P_{\text{free}}}{k_{\text{D}} + P_{\text{free}}}$$

binding to *n* independent and identical binding sites

$$D + n \cdot P_{\text{free}} \rightleftharpoons DP_{\text{n}} \qquad K_{\text{n}} = \frac{D_{\text{free}} \cdot P_{\text{free}}^{\text{n}}}{DP_{\text{n}}} \qquad \nu_{\text{n}} = \frac{n \cdot P_{\text{free}}^{\text{n}}}{K_{\text{n}} + P_{\text{free}}^{\text{n}}}$$

or divided by n

$$\theta = \frac{P_{\text{free}}^{\text{n}}}{K_n + P_{\text{free}}^{\text{n}}}$$

All or none binding (very high cooperativity)

$$M + nL \rightleftarrows ML_n$$

The equation for the equilibrium constant (K_n) is: $$K_n = \frac{[ML_n]}{[M][L]^n}$$

For n binding sites: $$\nu = \frac{n[ML_n]}{[M] + [ML_n]}$$

$$\nu = \frac{nK_n[M][L]^n}{[M] + K_n[M][L]^n} \qquad \text{divide by [M]}$$

$$\nu = \frac{nK_n[L]^n}{1 + K_n[L]^n} \qquad \text{divide by 1/n}$$

Remember that the fractional saturation is Y and Y = ν/n, so:

$$Y = \frac{K_n[L]^n}{1 + K_n[L]^n} \qquad \text{or} \qquad \theta = \frac{P_{\text{free}}^{\text{n}}}{K_n + P_{\text{free}}^{\text{n}}}$$

Binding to *n* identical binding sites

$$\nu_1 = \frac{P_{\text{free}}}{P_{\text{free}} + K_{\text{D}}}$$

binding to a single binding site

$$\nu_{\text{n}} = \frac{n \cdot P_{\text{free}}}{k_{\text{D}} + P_{\text{free}}}$$

binding to *n* independent and identical binding sites

$$\nu_n = \frac{n\ P_{\text{free}}^{\text{n}}}{K_n + P_{\text{free}}^{\text{n}}}$$

strong cooperative binding to *n* identical binding sites, with $K_{\text{n}} = (k_{\text{d}})^{\text{n}}$

$$\nu_{\text{n}} = \frac{n \cdot P_{\text{free}}^{\alpha_{\text{H}}}}{K^{\alpha_{\text{H}}} + P_{\text{free}}^{\alpha_{\text{H}}}}$$

approximation for cooperative binding to *n* identical binding sites, α_{H} Hill coefficient

$$\theta = \frac{P_{\text{free}}^{\alpha_{\text{H}}}}{K^{\alpha_{\text{H}}} + P_{\text{free}}^{\alpha_{\text{H}}}}$$

Hill coefficient and Hill plot

$$\theta = \frac{L_{\text{free}}^{\alpha_H}}{K^{\alpha_H} + L_{\text{free}}^{\alpha_H}}$$

approximation for cooperative binding to *n* identical binding sites, α_H HiIl coefficient
L_{free} is free ligand

The Hill α_H coefficient characterizes the degree of cooperativitiy. It varies from 1 (non-cooperative vinding) to n (the total number of bound ligands)

$\alpha_H > 1$, the system shows positive cooperativity
$\alpha_H = n$, the cooperativity is infinite
$\alpha_H = 1$, the system is non-cooperative
$\alpha_H < 1$, the system shows negative cooperativity

The Hill coefficient and the 'average' K_d can be obtained from a Hill plot, which is based on the transformation of the above equation

Hill coefficient and Hill plot

$$\theta = \frac{L_{\text{free}}^{\alpha_{\text{H}}}}{K^{\alpha_{\text{H}}} + L_{\text{free}}^{\alpha_{\text{H}}}}$$

α_{H} Hill coefficient
L_{free} is free ligand
K average microscopic binding constan

rearrange the terms to get

$$\frac{L_{\text{free}}^{\alpha_{\text{H}}}}{K^{\alpha_{\text{H}}}} = \frac{\theta}{1-\theta}$$

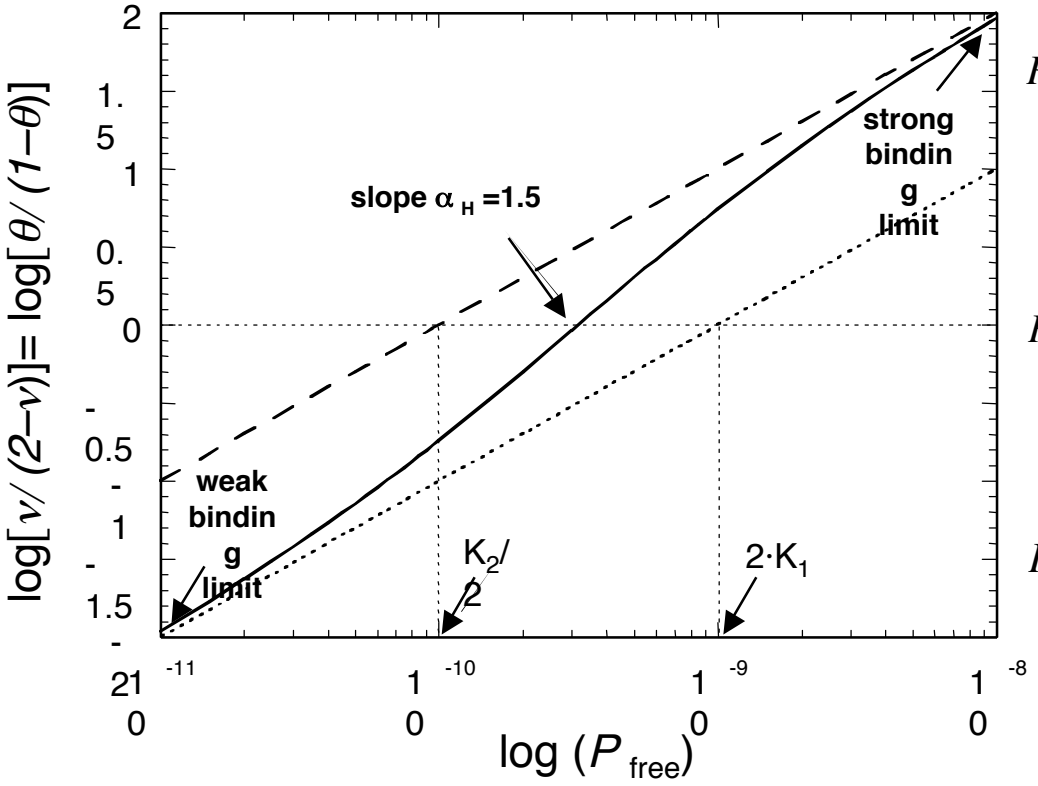
which yields the Hill equation

$$\log\left(\frac{\theta}{1-\theta}\right) = \alpha_{\text{H}} \log L_{\text{free}} - \log K^{\alpha_{\text{H}}}$$

Visualisation of binding data - Hill plot

$$\nu_n = \frac{n \cdot P_{free}^{\alpha_H}}{K^{\alpha_H} + P_{free}^{\alpha_H}}$$

$$\nu_2 = \frac{K_2 \cdot P_{free} + 2 \cdot P_{free}^2}{K_1 \cdot K_2 + K_2 \cdot P_{free} + P_{free}^2} \Leftrightarrow \frac{\nu_2}{2-\nu_2} = \frac{\theta}{1-\theta} = \frac{K_2 \cdot P_{free} + 2 \cdot P_{free}^2}{2 \cdot K_1 \cdot K_2 + K_2 \cdot P_{free}}$$



$$P_{free} \to \infty \Rightarrow \log\left(\frac{\nu_2}{2-\nu_2}\right) = \log\left(P_{free}\right) - \log\left(\frac{K_2}{2}\right)$$

$$P_{free} \approx K \Rightarrow \log\left(\frac{\nu_2}{2-\nu_2}\right) = \alpha_H \cdot \log\left(P_{free}\right) - \alpha_H \cdot \log\left(K\right)$$

$$P_{free} \to 0 \Rightarrow \log\left(\frac{\nu_2}{2-\nu_2}\right) = \log\left(P_{free}\right) - \log\left(2K_1\right)$$

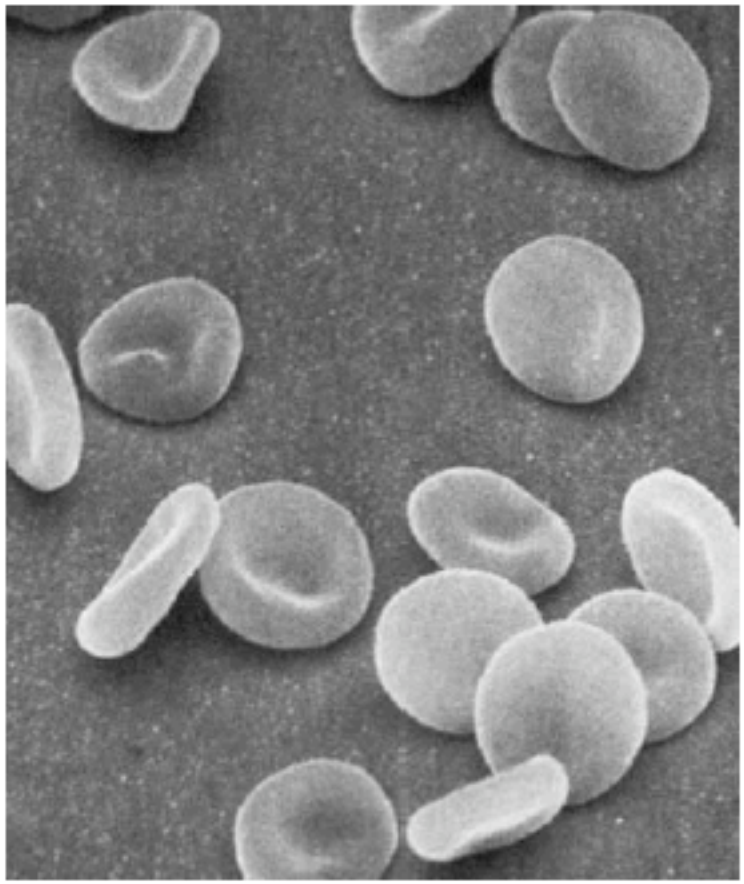
Why isn't the Hill plot linear?

- When cooperativity is not complete (i.e., $n_h < N$), the Hill plot is not linear.
- At the extremes of [L], the line has a slope of ~1.0.
- At low ligand concentrations, there is no cooperativity. Thus the Hill plot will represent single-site binding (binding of the first ligand molecule).
- At high ligand concentrations, all sites are filled but one. Thus this region of the Hill plot should also represent single-site binding for the last ligand.

Biological Uses of Cooperativity and Allostery

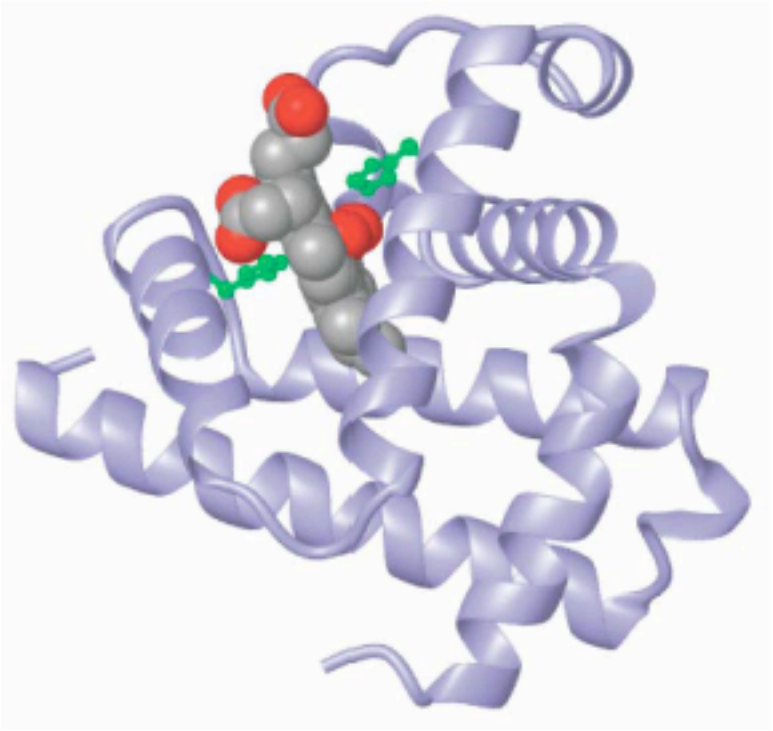
Hemoglobin: Efficient Ligand Delivery

- Hemoglobin binds O_2 reversibly under different partial pressures
- Why make hemoglobin cooperative?
- Positive cooperativity gives all or none behavior. Thus, hemoglobin saturates at about the same O_2 concentration as myoglobin, but releases essentially all of its O_2 cargo at much higher partial pressure of O_2.



Each erythrocyte contains ~300 million hemoglobin molecules.

Heme Proteins: Myoglobin and Hemoglobin

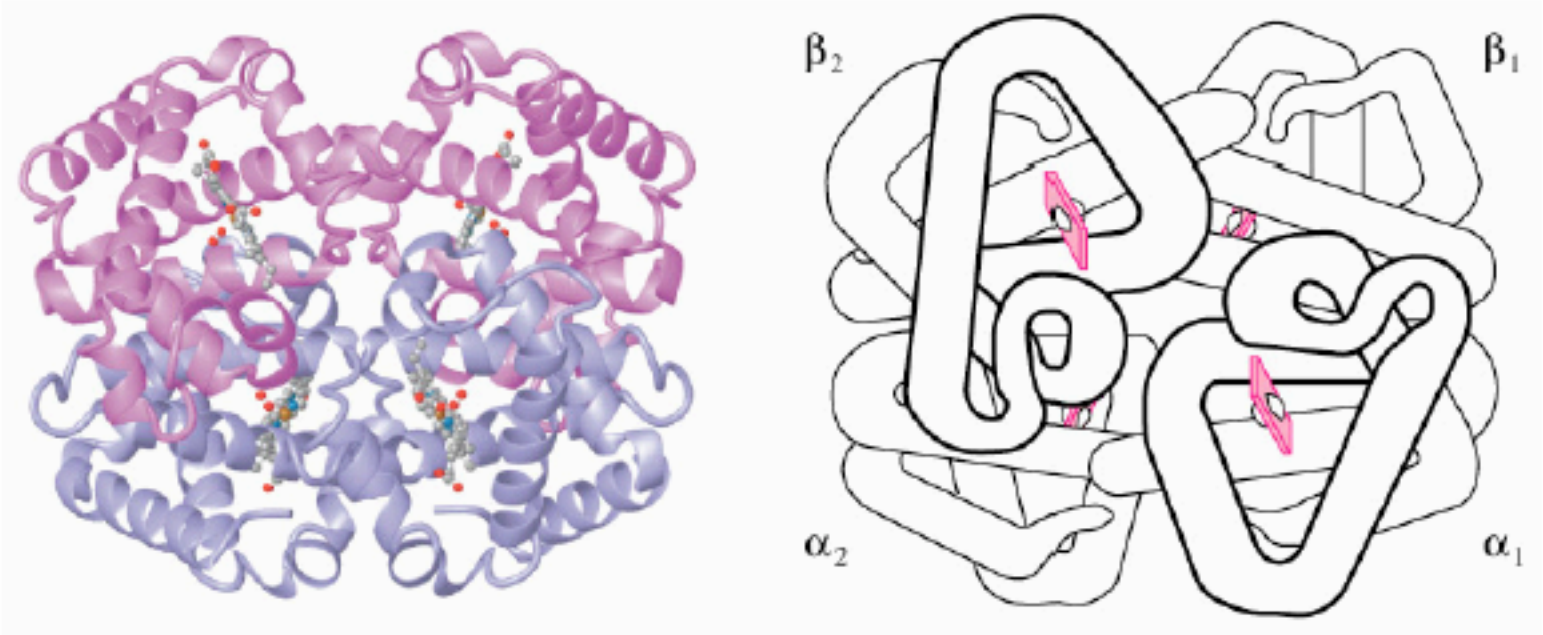


Myoglobin

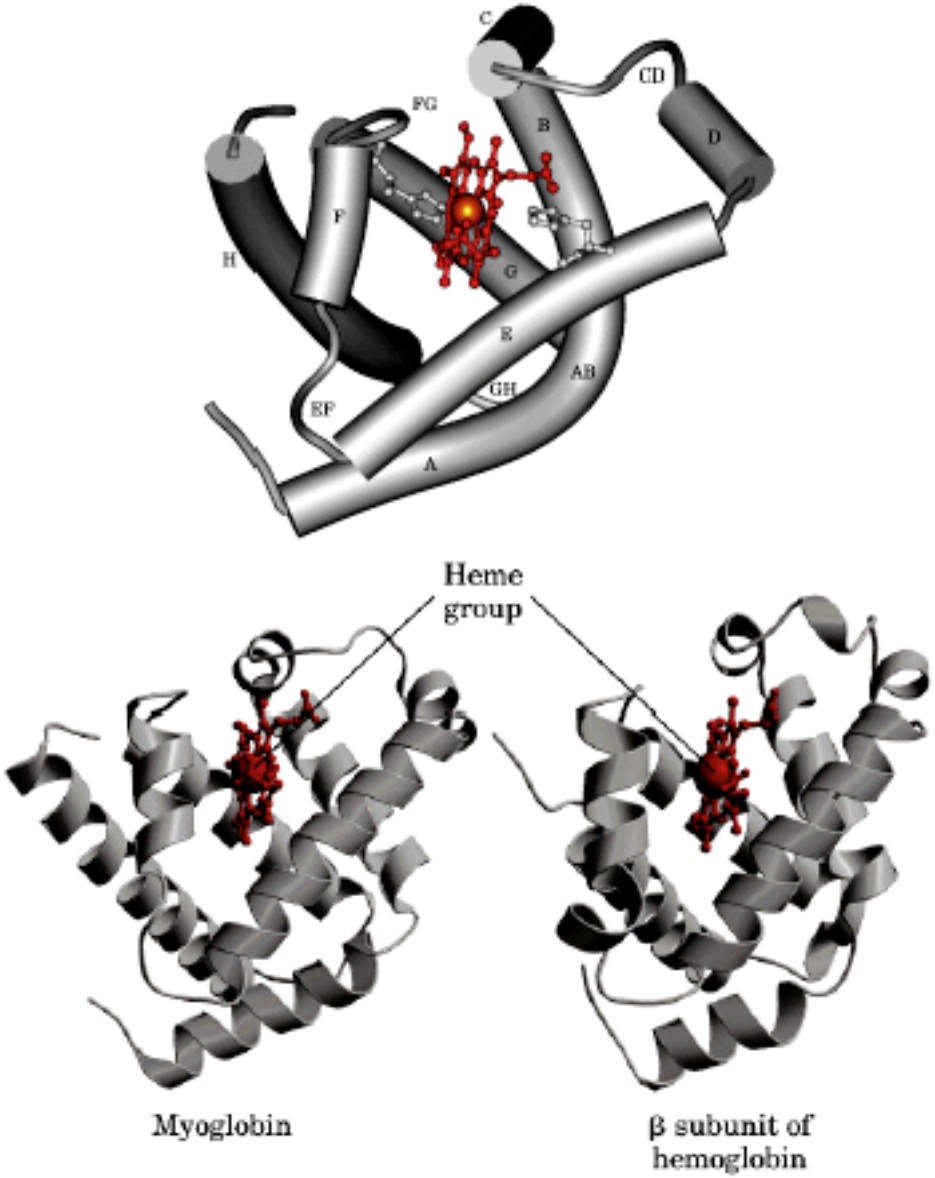
- Compact, globular protein (75% α-helix).
- Single polypeptide chain of 153 residues mw ~16.7 kDa.
- Covalently bound heme group.
- Oxygen storage protein of muscle, prevalent in diving mammals.

Hemoglobin

- Tetramer composed of two α-subunits and two β-subunits (α2β2 tetramer).
- The α-subunit is 141 residues and the β-subunit is 146 residues.
- Each polypeptide chain is structurally similar to myoglobin.
- Each polypeptide chain contains a covalently bound heme group.

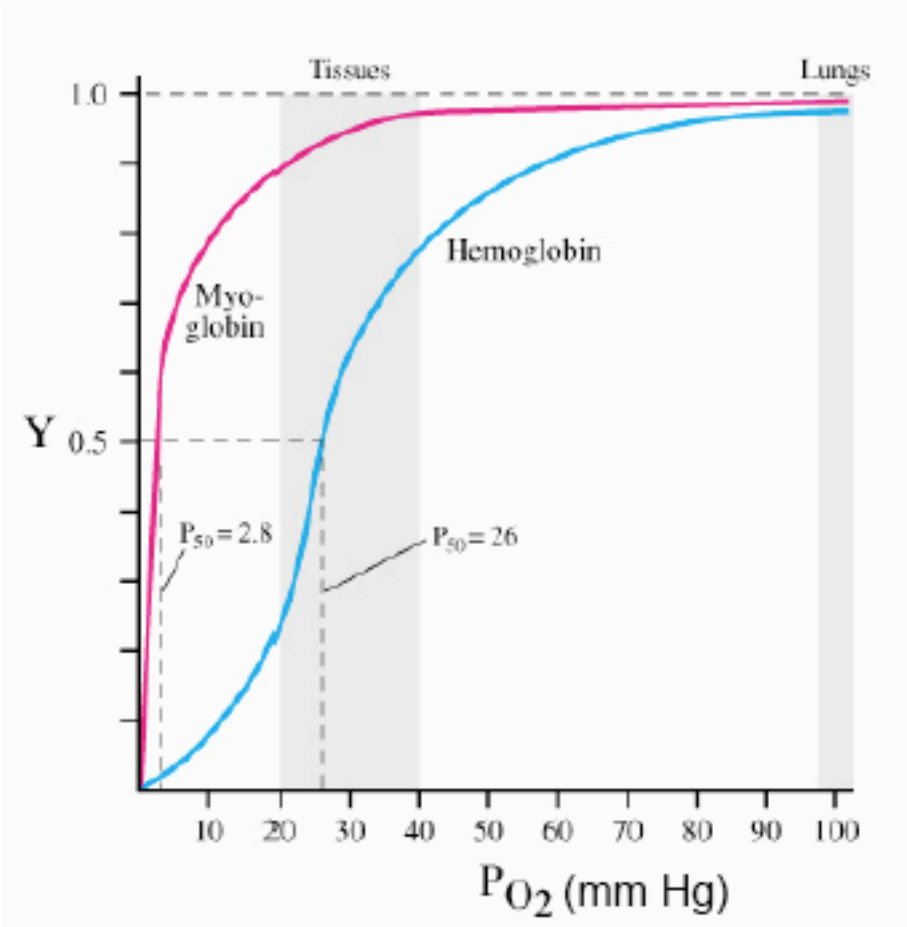


Structural Similarities between Myoglobin and Hemoglobin



- Each subunit of hemoglobin has a tertiary fold that is similar to myoglobin.
- Myoglobin is composed of eight helical segments (shown on the left as cylinders) lettered A–H. The loops are labeled with the letters of the helices that they connect.
- The histidine that coordinates the heme iron in myoglobin is His93, which is also sometimes referred to as His F8, which stands for the eighth amino acid in helix F.

The oxygen binding curves for hemoglobin and myoglobin are significantly different.

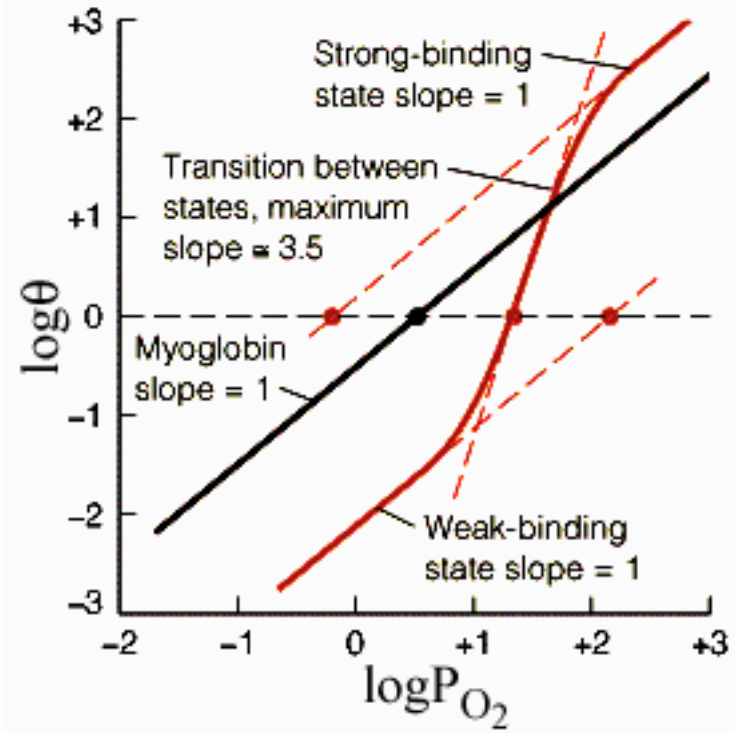


The free oxygen is expressed as the partial pressure of oxygen (P_{O_2}).

Myoglobin vs. Hemoglobin

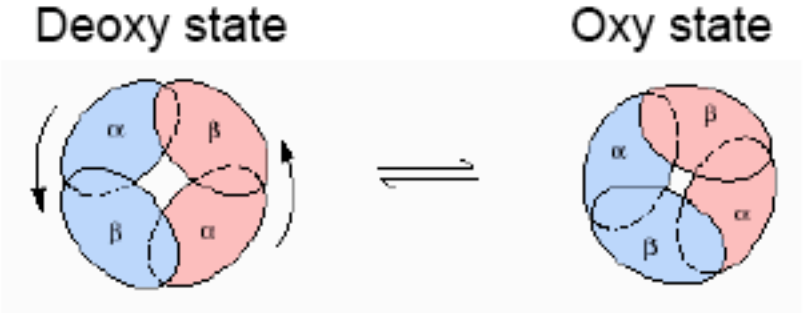
- Hemoglobin binds O_2 less tightly.
- Hemoglobin displays cooperativity (*i.e.* binding of one O_2 molecule increases the affinity for subsequent O_2 binding).
- Hemoglobin saturates at about the same O_2 concentration as myoglobin, but releases essentially all of its O_2 cargo at much higher partial pressure of O_2 than myoglobin.

Hill Plots for Oxygen Binding to Hemoglobin and Myoglobin



- At low P_{O_2} , the Hill plot has a slope = 1 and corresponds to the weak binding state (large P_{50})
- As binding progresses, the curve switches over to approach another parallel straight line that describes the strong binding state (small P_{50}).
- The transition between binding states is clear for cooperative (Hb) and non-cooperative (Mb) systems.

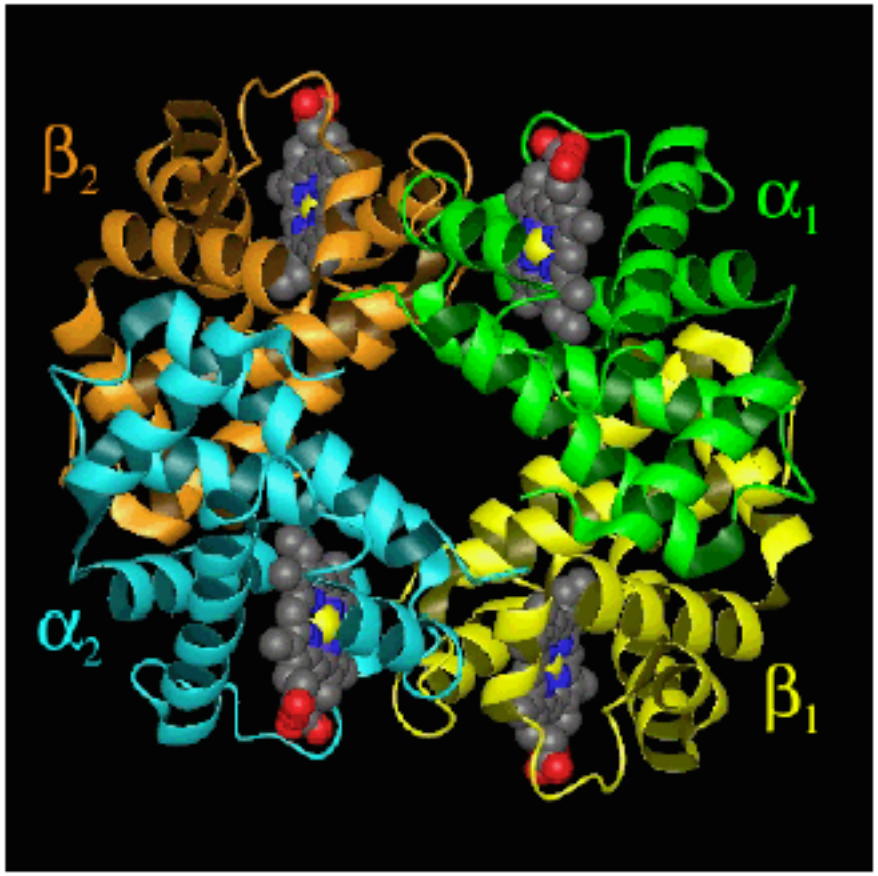
O_2 binding to the heme effects the entire hemoglobin structure.



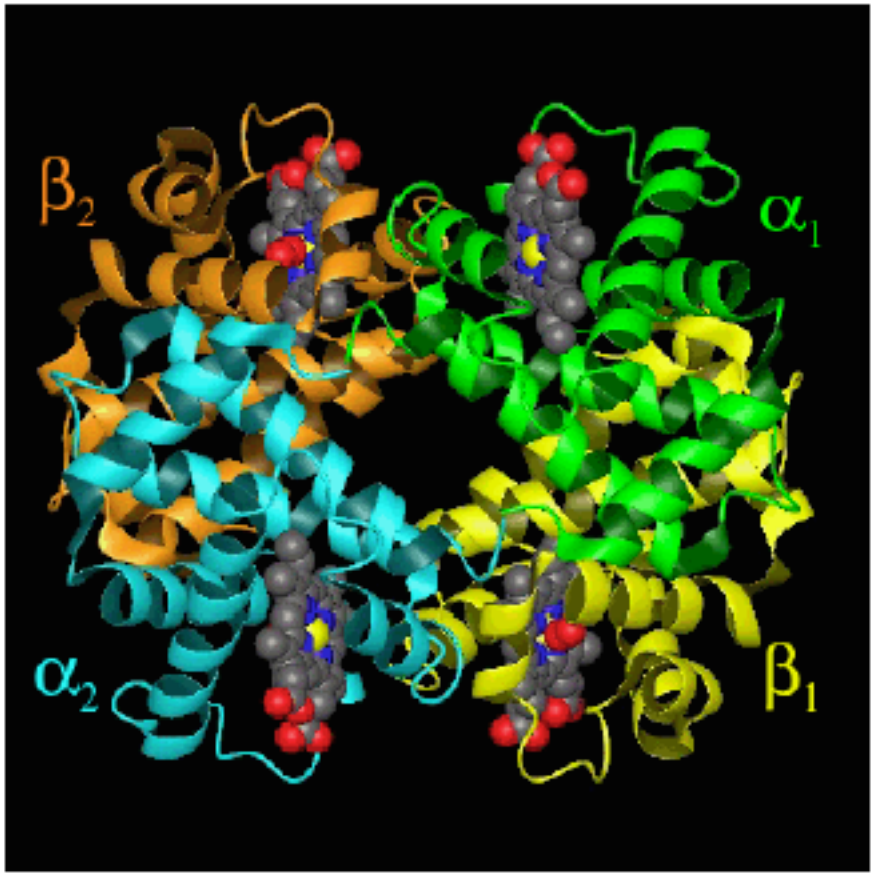
- O_2 binding causes a series of shifts in all subunits, one αβ pair rotates and slides with respect to the other pair.
- There is a change in the heme structure upon binding O_2.
- Since His F8 is covalently attached to the heme, all of helix F shifts.
- The reorganization of helix F alters the tertiary structure, which in turn alters the quaternary structure- all 4 subunits behave as a single cooperative structural unit.
- There are changes in the packing of hydrophobic side chains and changes in the pairing of charged side chains.
- The change in conformation of hemoglobin from the T to the R state increases the O_2 affinity at ALL sites.

Structures of deoxygenated and oxygenated hemoglobin.

Deoxy-T (tense) state

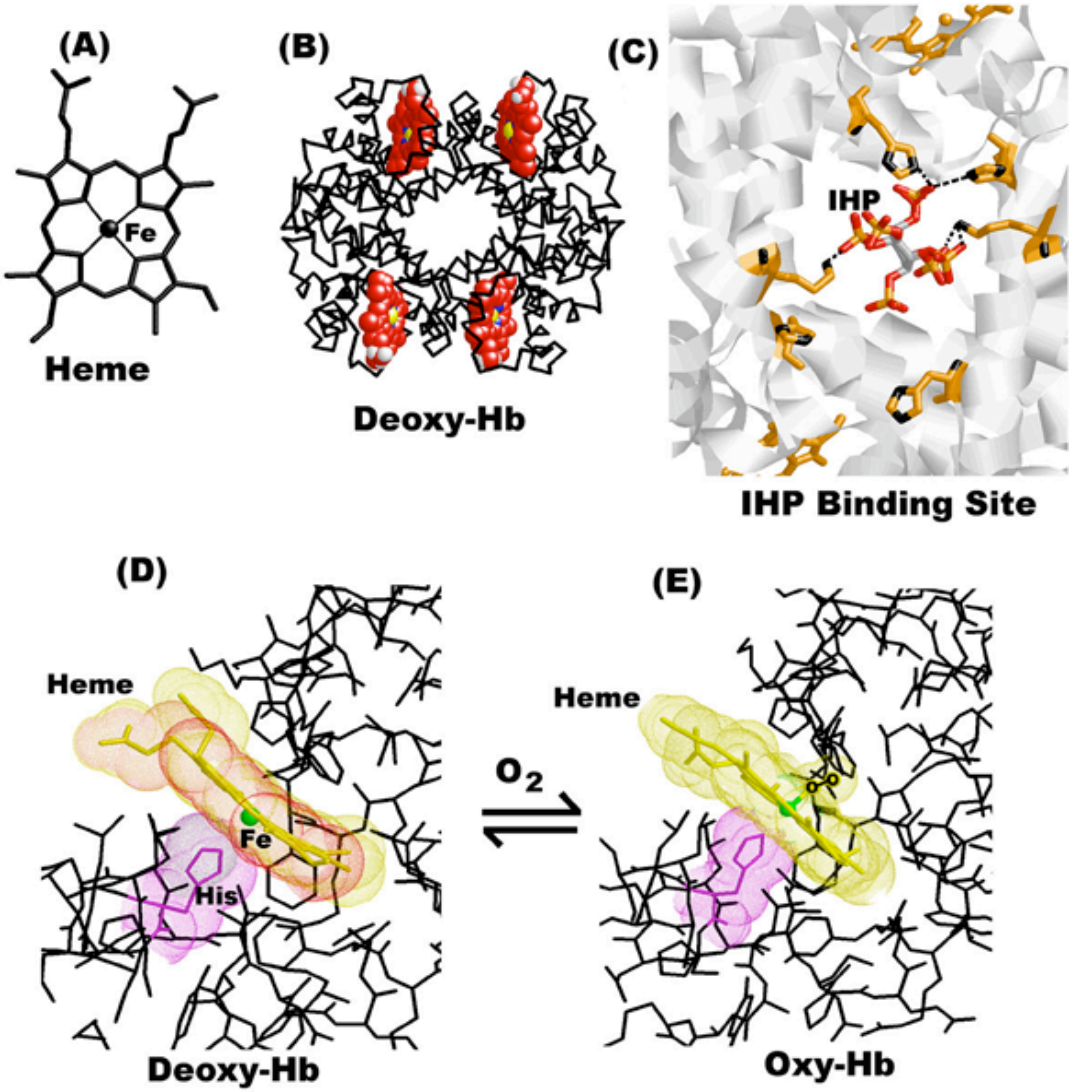


Oxy- R (relaxed) state

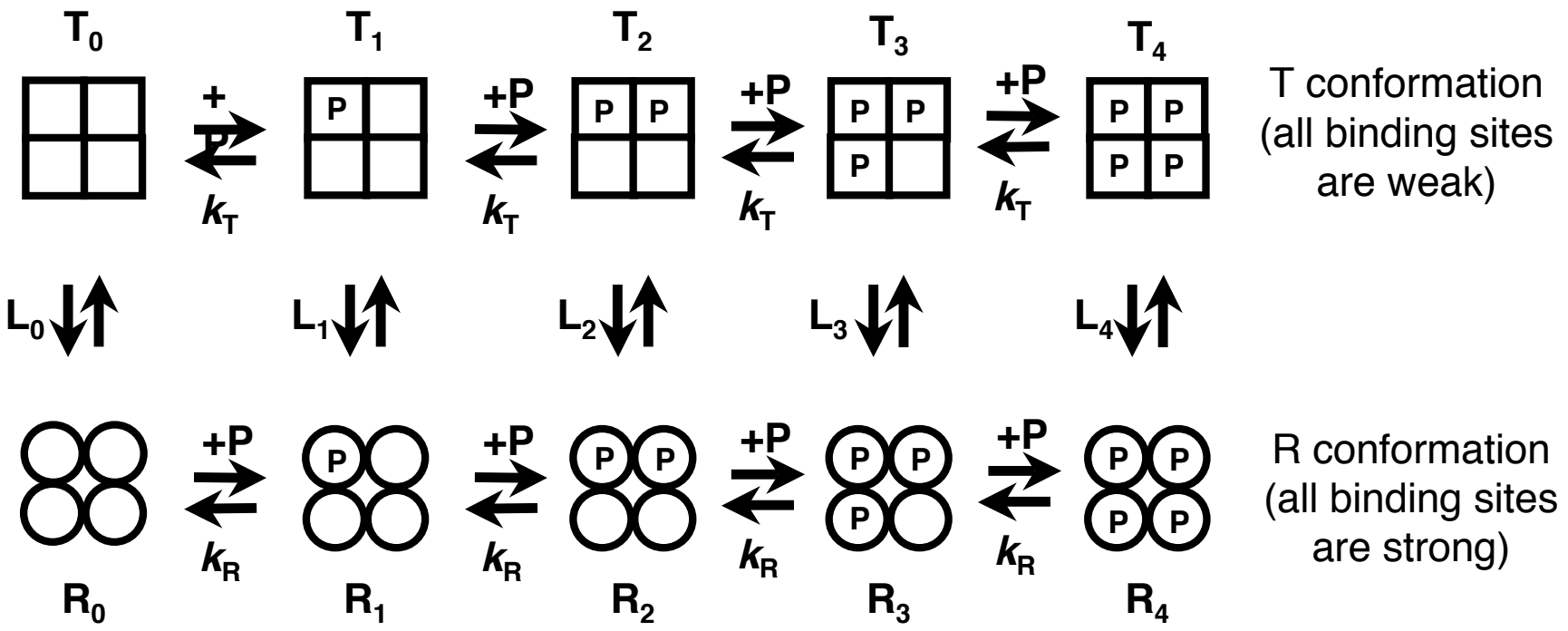


Hemoglobin Gallery of still pictures and animations by Dr. John Lukin
http://www.andrew.cmu.edu/user/jl2p/Hb_html/gallery.html

Binding of dioxygen to hemoglobin

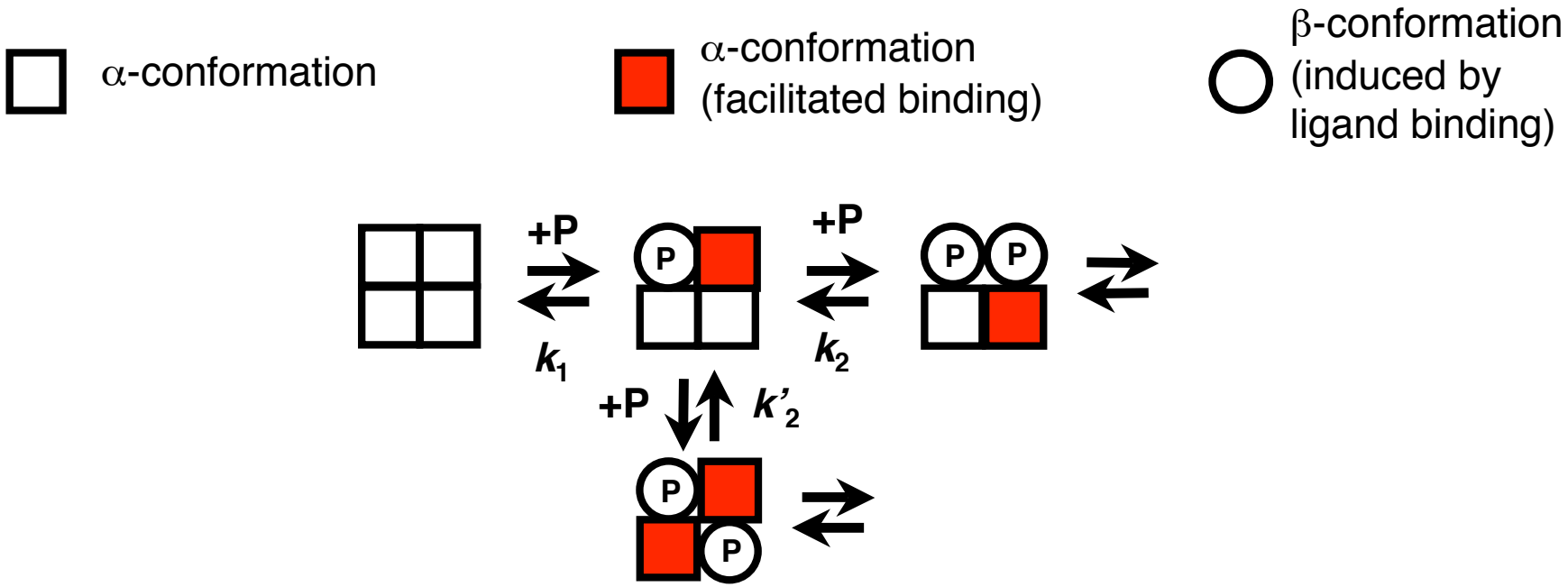


The Monod-Wyman-Changeau (MWC) model for cooperative binding



- **in the absence of ligand P the the T conformation is favored**
- **the ligand affinity to the R form is higher, i. e. the dissociation constant $k_R < k_T$.**
- **all subunits are present in the same confomation**
- **binding of each ligand changes the T<->R equilibrium towards the R-Form**

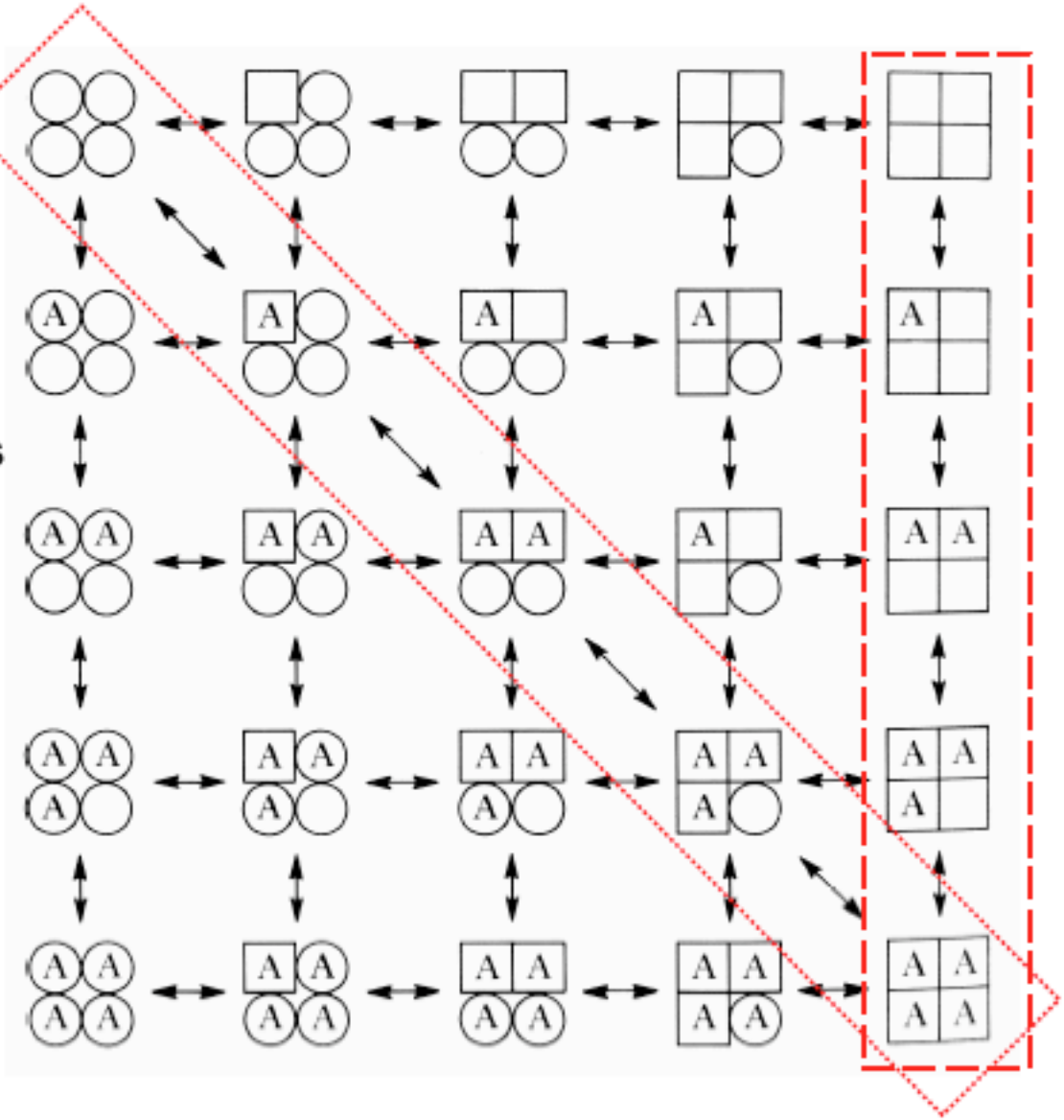
The Koshland-Nemethy-Filmer (KNF) model for cooperative binding



- **Binding of ligand P induces a conformation change in the subunit to which it binds from the α into the β-conformation ("induced fit").**
- **The bound ligand P facilitates the binding of P to a nearby subunit in the α-conformation (red), i. e. the dissociation constant $k_2 < k'_2$.**
- **subunits can adopt a mixture of α–β confomations.**

A more general allosteric scheme…

- This scheme allows the individual subunits to take on either of two conformational forms, regardless of the number of ligands that are bound.
- For a four-subunit protein, this allow 25 different combinations.
- The MWC model is a limiting case of this scheme involving only the species enclosed by the dashed rectangle.
- The sequential scheme involves the forms enclosed by the diagonal dotted rectangle.



Why are multistate models needed?

- Neither the KNF nor the MWC model exactly explains the allosteric behavior of proteins, including hemoglobin. Consequently, more complex models have been devised.

- Most such models retain the MWC concept of a concerted switch in conformation, but involve more than two states for the entire molecule. This is because the MWC model uses only a few parameters.

- However, when observations cannot be accommodated by the MWC model, more complicated schemes are considered.

Summary

- Thermodynamic relation between ΔG und K_D
- Stoichiometry of binding
- Determination of the dissociation constant for simple systems
- Adair equation for a general description of binding
- Binding to n binding sites
- Visualisation of binding curves by Scatchard and Hill plots
- Cooperativity of binding (MWC and KNF model)