Increasing complexity of binding

all binding sites are
equivalent and independent

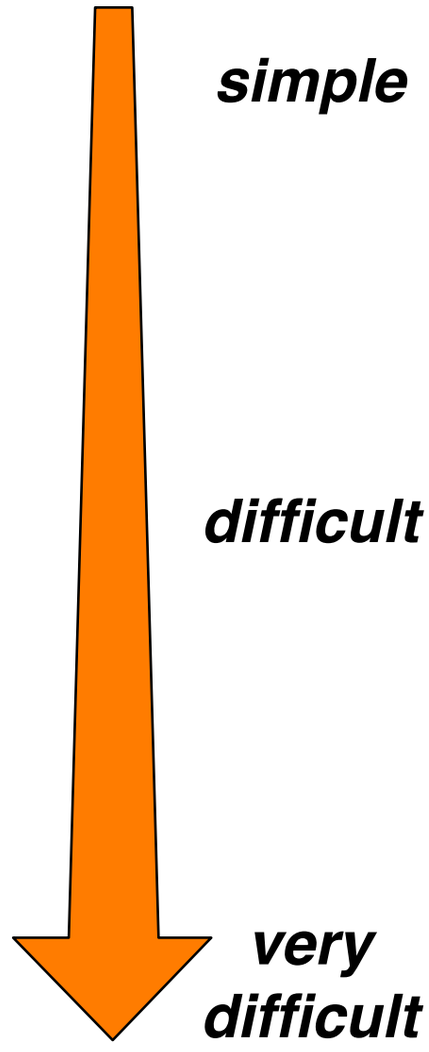
cooperativity ↙ ↘ ***heterogeneity***

all binding sites are
equivalent and not independent

all binding sites are
independent but not equivalent

heterogeneity ↘ ↙ ***cooperativity***

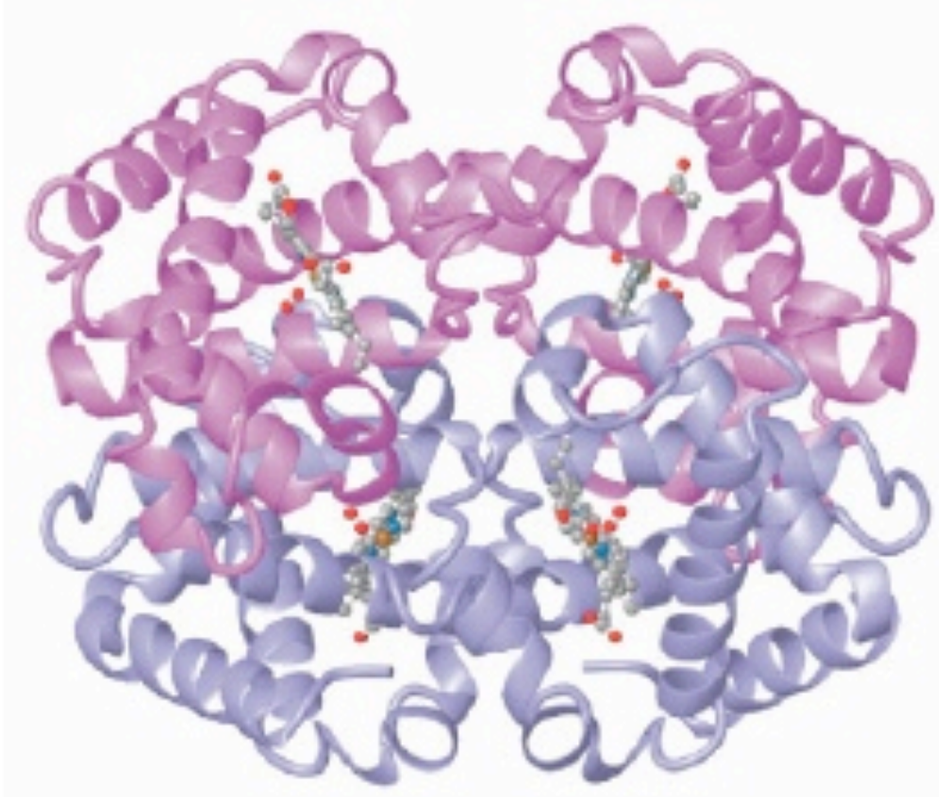
all binding sites are
not equivalent and not independent

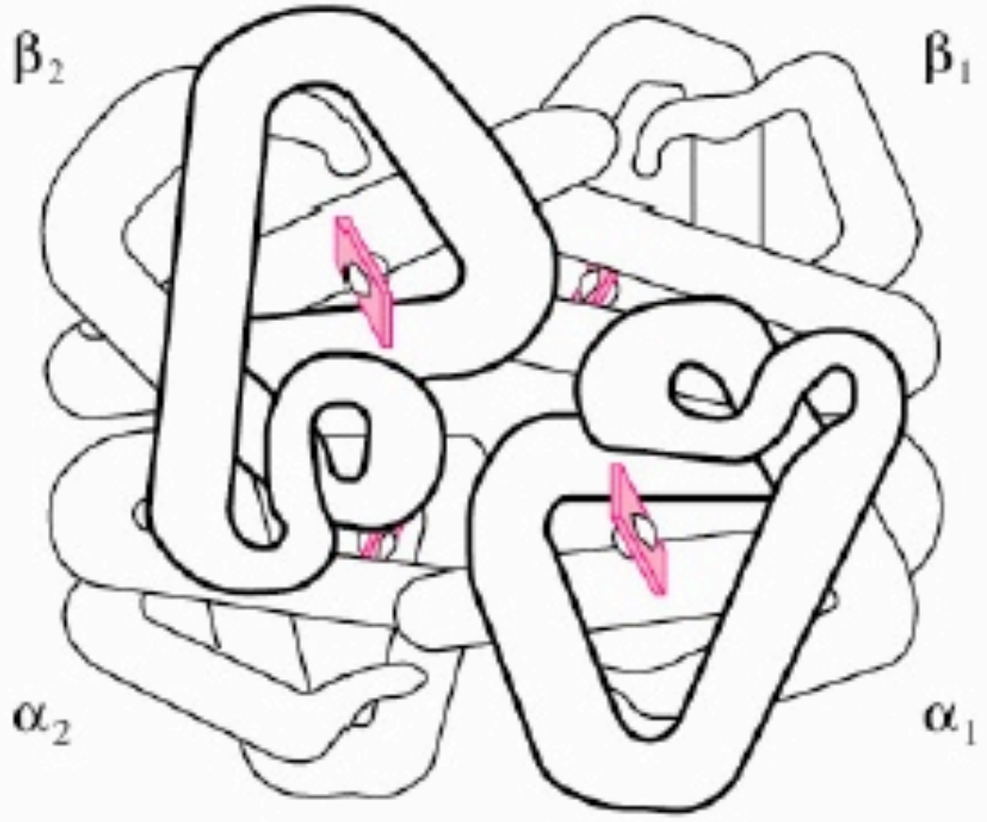


An example for complex binding: oxygen binding to hemoglobin

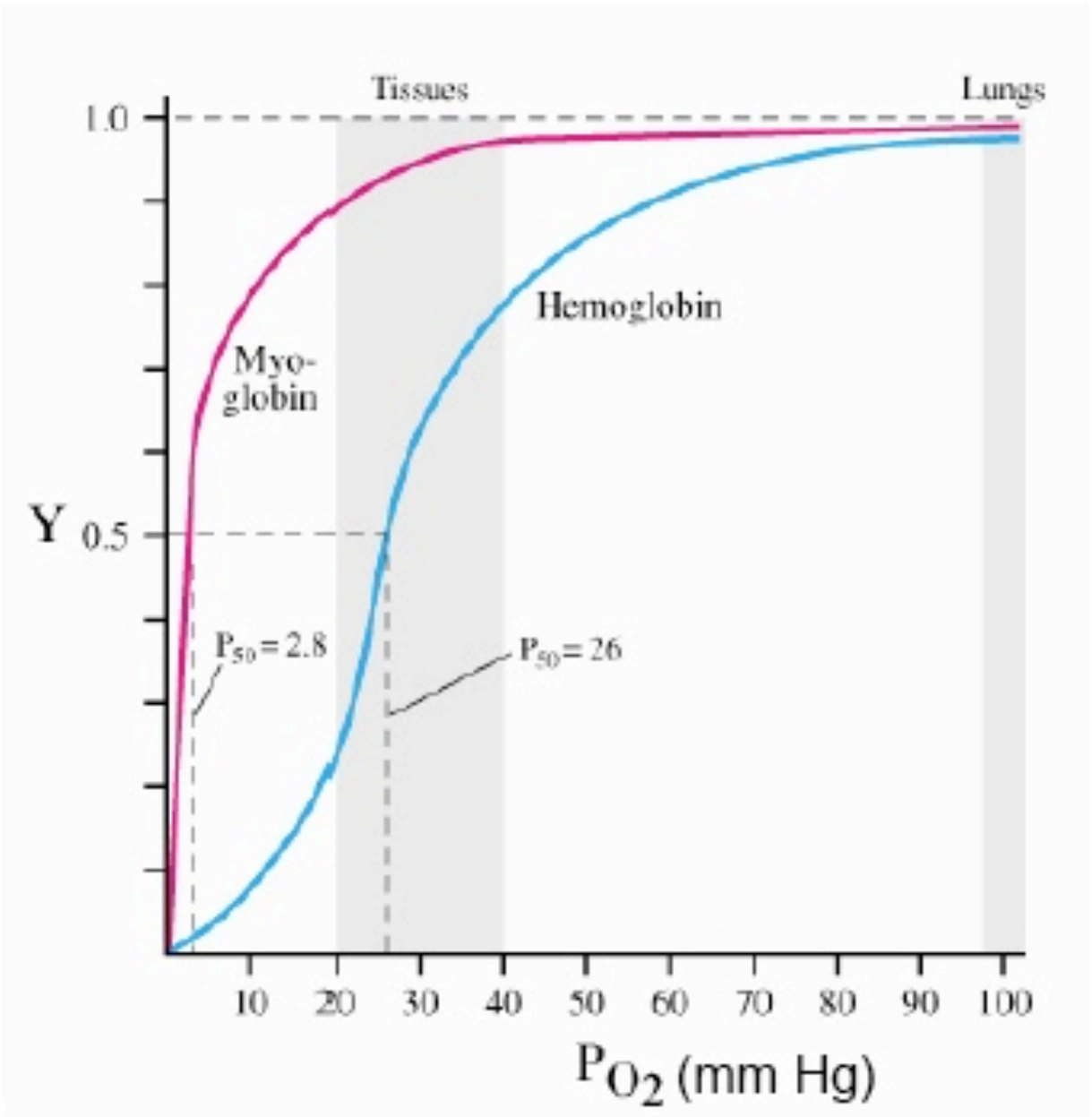
Hemoglobin

- Tetramer composed of two α-subunits and two β-subunits ($\alpha 2 \beta 2$ 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.





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.

Definition of the degree of binding ν

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

degree of binding ν

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

ν for *n* binding sites (Adair equation)

Expression degree of binding ν for four sites

$$DP_1 <-> D + P; \quad K_1 = D \cdot P / DP_1; \quad DP_1 = D \cdot P / K_1$$
$$DP_2 <-> D + 2P; \quad K_2 = D \cdot P^2 / DP_2; \quad DP_2 = D \cdot P^2 / K_2$$
$$DP_3 <-> D + 3P; \quad K_3 = D \cdot P^3 / DP_3; \quad DP_3 = D \cdot P^3 / K_3$$
$$DP_4 <-> D + 4P; \quad K_4 = D \cdot P^4 / DP_4; \quad DP_4 = D \cdot P^4 / K_4$$

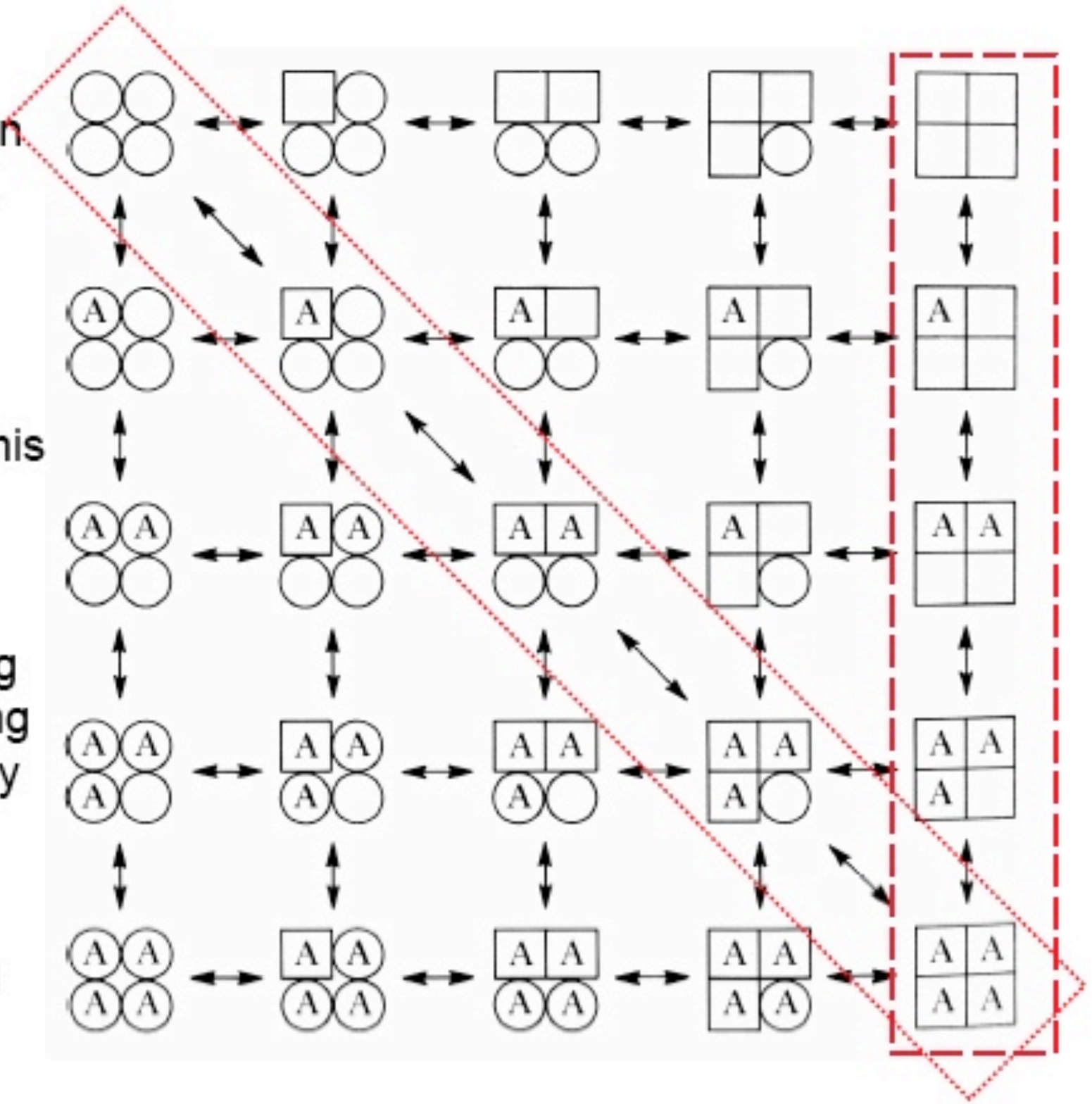
$$\nu_4 = \frac{\text{bound ligand}}{\text{macromolecule}} = \frac{DP_1 + 2DP_2 + 3DP_3 + 4DP_4}{D + DP_1 + DP_2 + DP_3 + DP_4}$$

$$\nu_4 = \frac{\frac{1}{K_1} \cdot P_{\text{free}}^1 + \frac{2}{K_2} \cdot P_{\text{free}}^2 + \frac{3}{K_3} \cdot P_{\text{free}}^3 + \frac{4}{K_4} \cdot P_{\text{free}}^4}{1 + \frac{1}{K_1} \cdot P_{\text{free}}^1 + \frac{1}{K_2} \cdot P_{\text{free}}^2 + \frac{1}{K_3} \cdot P_{\text{free}}^3 + \frac{1}{K_4} \cdot P_{\text{free}}^4}$$

$$\nu_4 = \frac{K_2K_3K_4 \cdot P_{\text{free}}^1 + 2K_1K_3K_4 \cdot P_{\text{free}}^2 + 3K_1K_2K_4 \cdot P_{\text{free}}^3 + 4K_1K_2K_3 \cdot P_{\text{free}}^4}{K_1K_2K_3K_4 + K_2K_3K_4 \cdot P_{\text{free}}^1 + K_1K_3K_4 \cdot P_{\text{free}}^2 + K_1K_2K_4 \cdot P_{\text{free}}^3 + K_1K_2K_3 \cdot P_{\text{free}}^4}$$

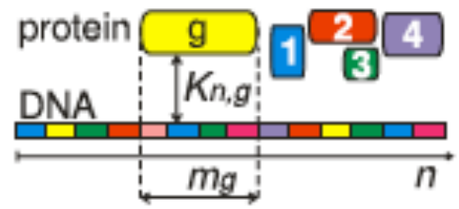
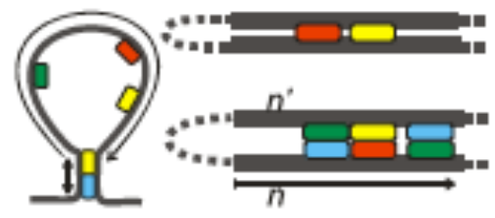
A more general allosteric scheme... (oxygen binding to hemoglobin)

- 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.



Complex binding equilibria for protein-DNA interactions

Table 1. Basic features of DNA–protein binding and lattice approaches.

Feature/method	Combinatorial method	Generating functions	Transfer matrices	Recurrent relations	Graphical representation
Sequence specificity	−	+	+	+	
Overlapping binding sites	+	+	+	+	
Multiprotein competition	−	−	+	+	
Contact interactions	+	+	+	+	
Long-range interactions	+	+	+	+	
Multilayer binding	−	+	+	−	
Short DNA loops	−	−	+	+	
Long DNA loops (e.g. promoter–enhancer)	+	−	+	−	

Teif, V. B. & Rippe, K. Statistical-mechanical lattice models for protein-DNA binding in chromatin. *J Phys Condens Matter* **22,** 414105 (2010).

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

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

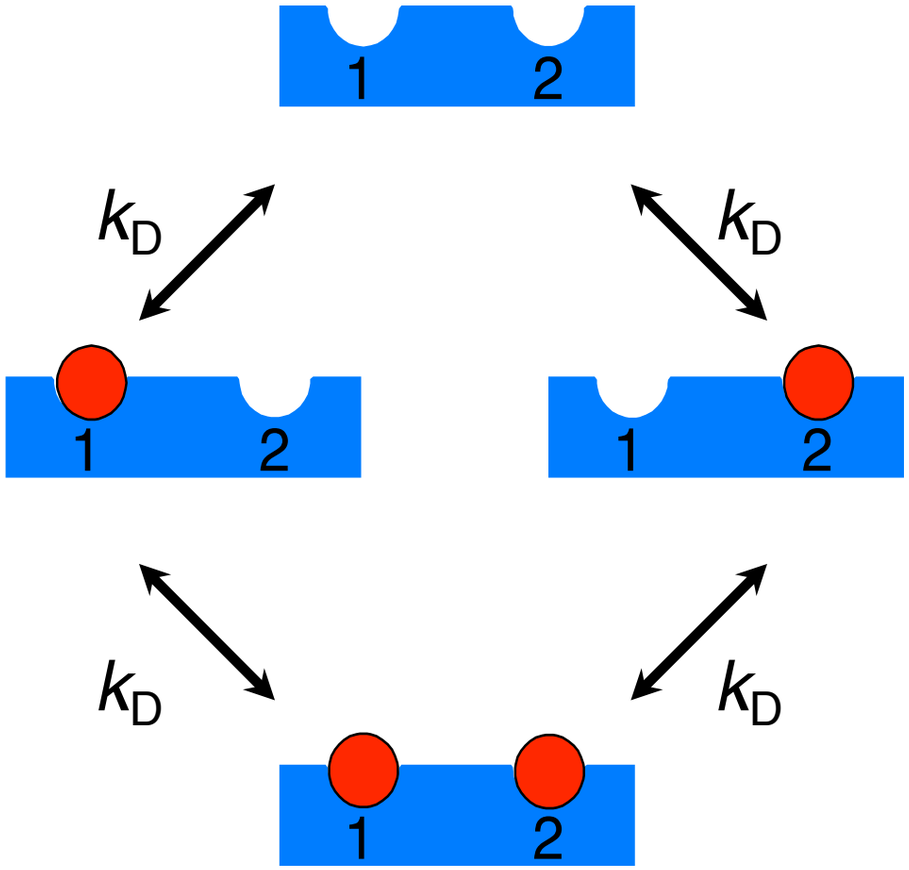
strong cooperative binding to *n* identical binding sites

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

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*

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

DP

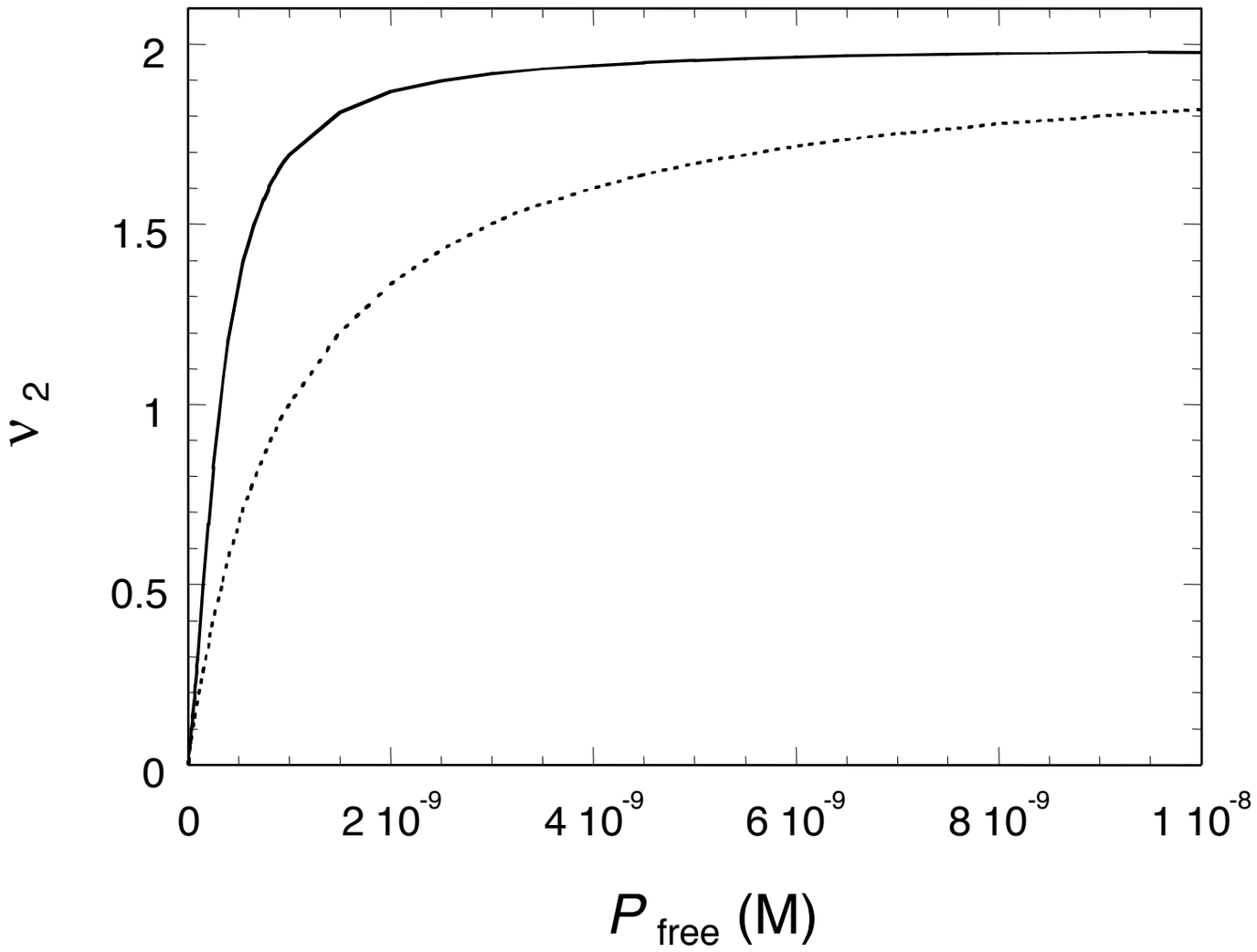
2 possibilities for the dissociation of DP_2

$$K_2 = 2k_d$$

DP_2

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

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)

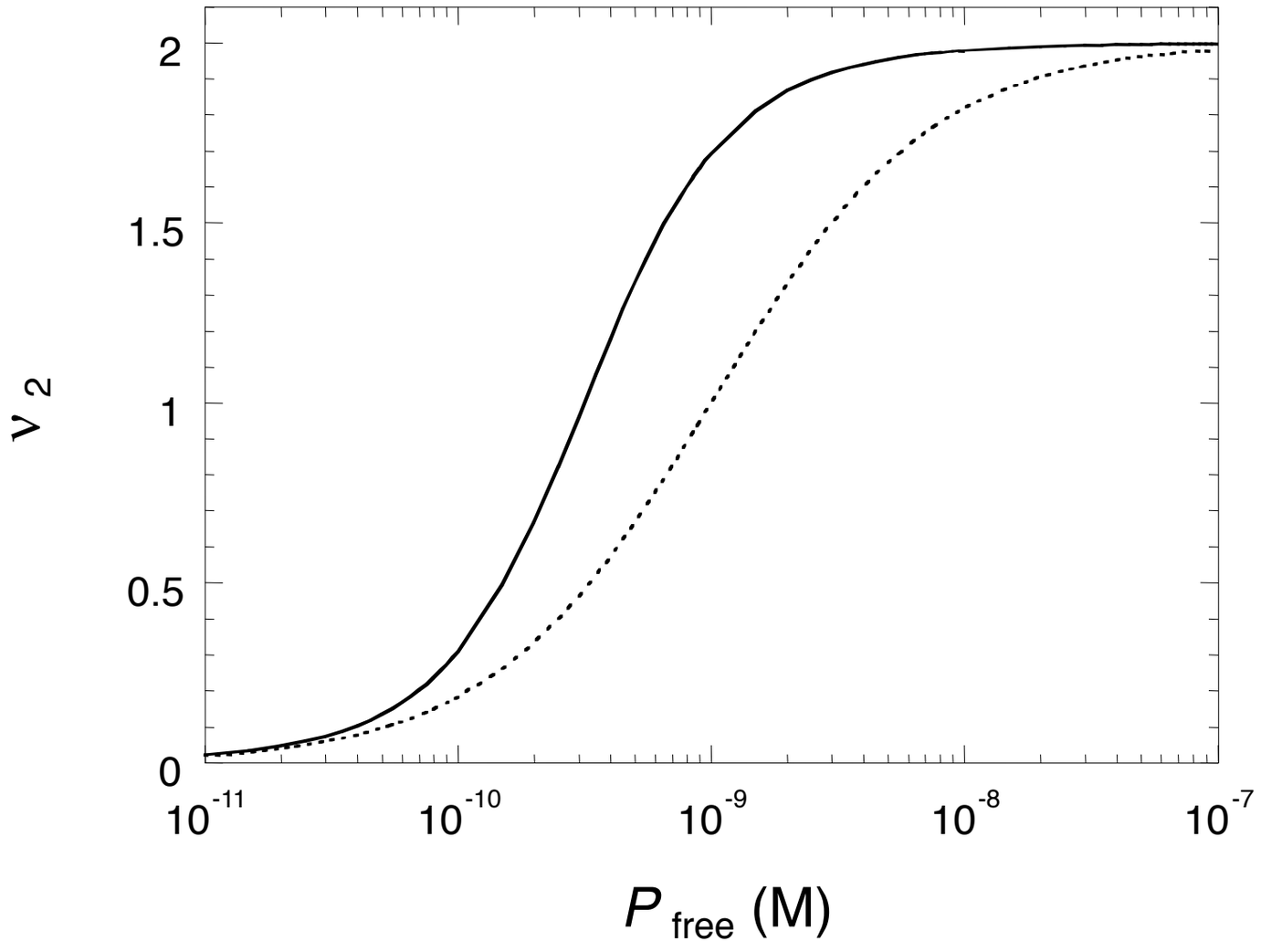
cooperative binding
microscopic binding constant
$k_D = 10^{-9}$ (M)

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

Adair equation

$$\nu_2 = \frac{K_2 P_{\text{free}} + 2P_{\text{free}}^2}{K_1 K_2 + K_2 P_{\text{free}} + P_{\text{free}}^2}$$

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 at least three orders of a ligand concentration
- Chemical potential μ is proportional to the logarithm of the concentration.

Binding to *n* identical binding sites

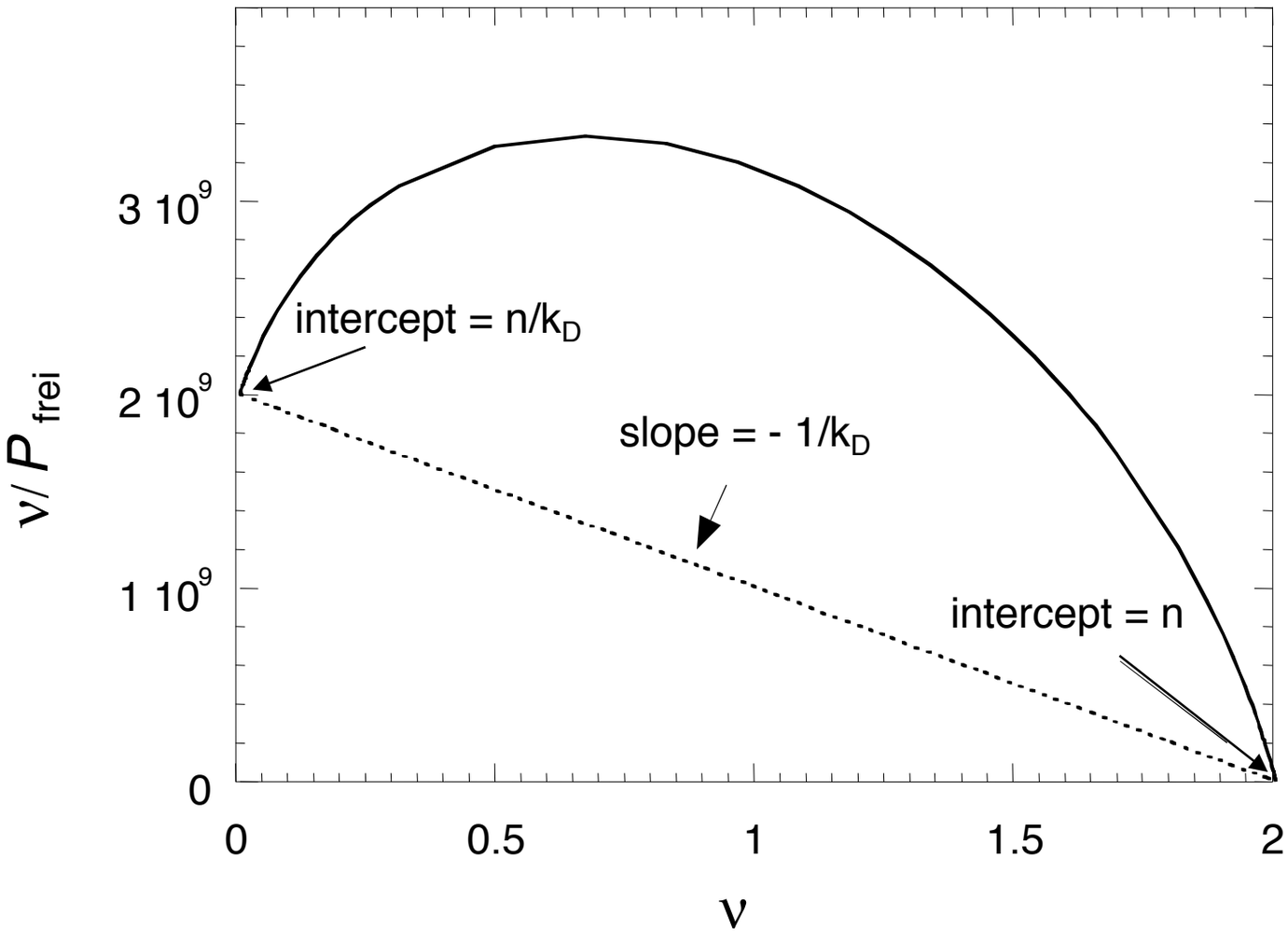
$$\nu_1 = \frac{P_{\text{free}}}{P_{\text{free}} + K_{\text{D}}}$$

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

Visualisation of binding data - Scatchard plot



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)

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

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

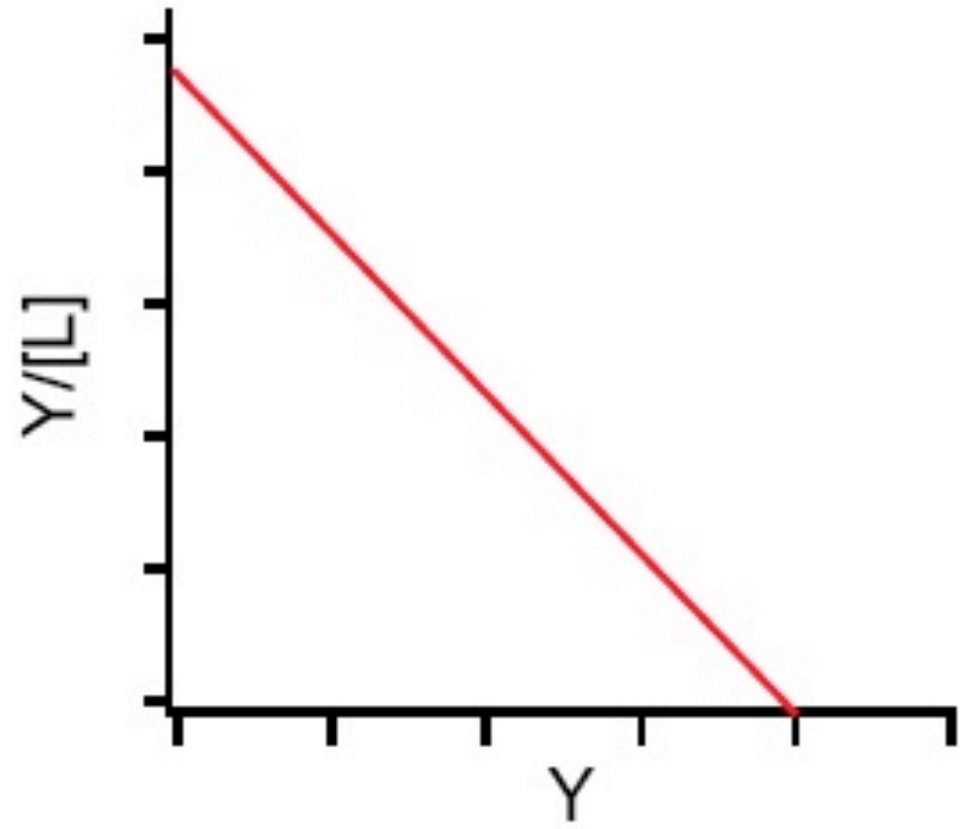
$$Y + Y[L]K_{eq} = [L]K_{eq}$$

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).

Scatchard plots

Scatchard plot is useful as a visualisation tool especially for displaying changes in n or K_d under different conditions or for identifying binding site heterogeneity of cooperativity (curved Scatchard plot)

Linearization is used to simplify analysis that would be more accurate using nonlinear regression programs. For example there are large variations in the error bars.

All or none binding (very high cooperativity)

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

for n binding sites,
"all or none" binding

$$\nu = \frac{n \cdot DP_{\text{n}}}{D + DP_{\text{n}}} \qquad \nu = \frac{[\text{bound ligand } P]}{[\text{macromolecule } D]}$$

$$\nu = \frac{n \cdot D \cdot P_{\text{free}}^{\text{n}} / K_n}{D + D \cdot P_{\text{free}}^{\text{n}} / K_n}$$

divide by D

$$\nu = \frac{n \cdot P_{\text{free}}^{\text{n}} / K_n}{1 + P_{\text{free}}^{\text{n}} / K_n} = \frac{n \cdot P_{\text{free}}^{\text{n}}}{K_n + P_{\text{free}}^{\text{n}}} \qquad \text{or} \qquad \theta = \frac{P_{\text{free}}^{\text{n}}}{K_n + P_{\text{free}}^{\text{n}}}$$

divided by 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 HiIl 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

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

α_H HIll coefficient
L_{free} is free ligand
K average microscopic binding constan

rearrange the terms to get

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

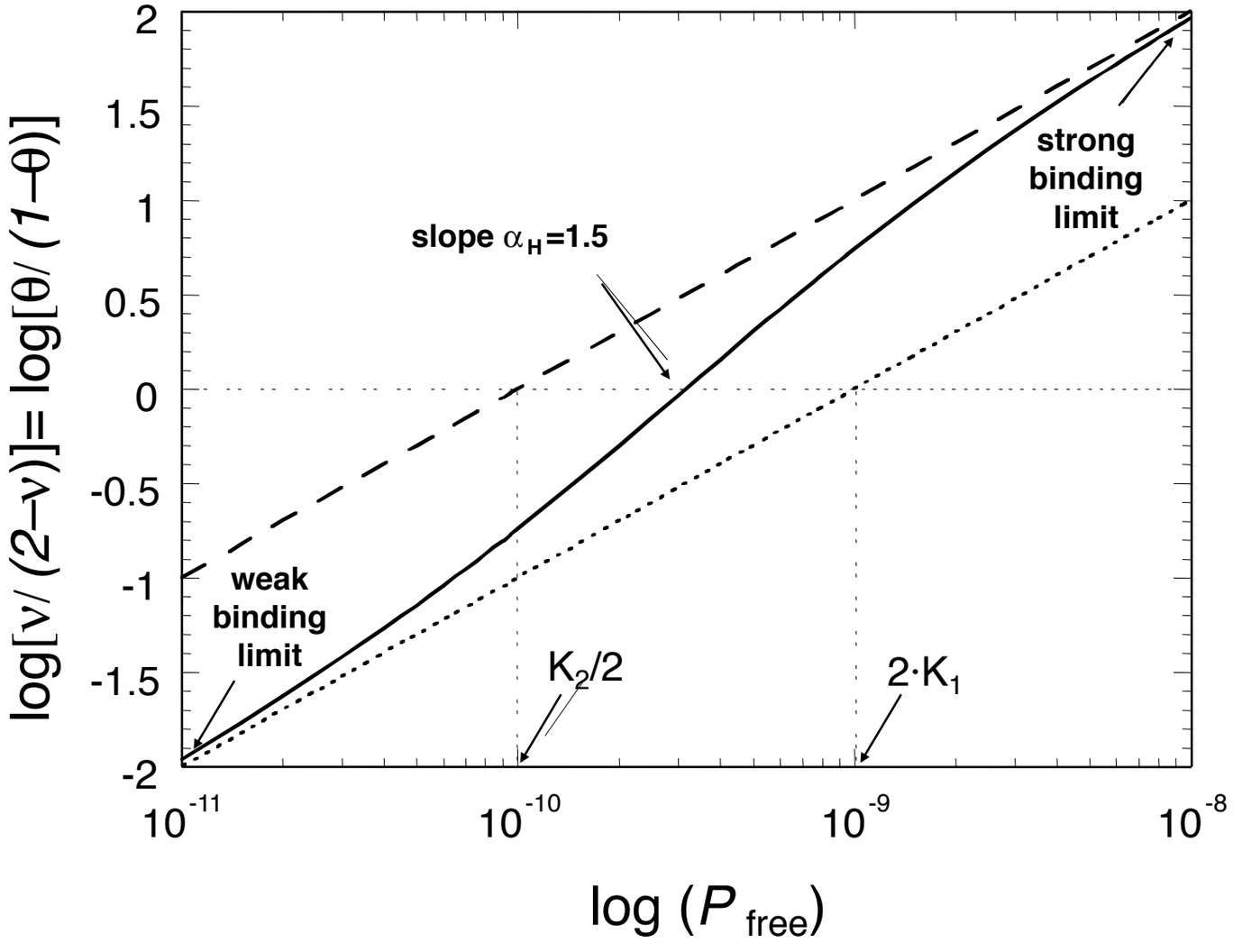
which yields the Hill equation

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

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.

Summary

- Players: DNA, proteins, solution
- Thermodynamic equilibrium: ΔG, K_D and K_B; $\Delta G = - R\,T \ln(K_D)$; $K_B = 1/K_D$
- Ways to look at the binding constant K:
  - $K = \exp(-\Delta G/RT)$
  - K = rate_binding / rate_dissociation
  - K = probability of binding
- Ways to visualize binding curves:
  - Linear (Langmuir) plot: $\nu = f(P_{free})$
  - Logarithmic plot: $\nu = f(\mathrm{Log}(P_{free}))$
  - Hill plot: $\mathrm{Log}(\theta/(1-\theta))/\mathrm{Log} = f(P_{free})$, $\theta = \nu/n$
  - Scatchard plot: $\nu/P_{free} = f(\nu)$

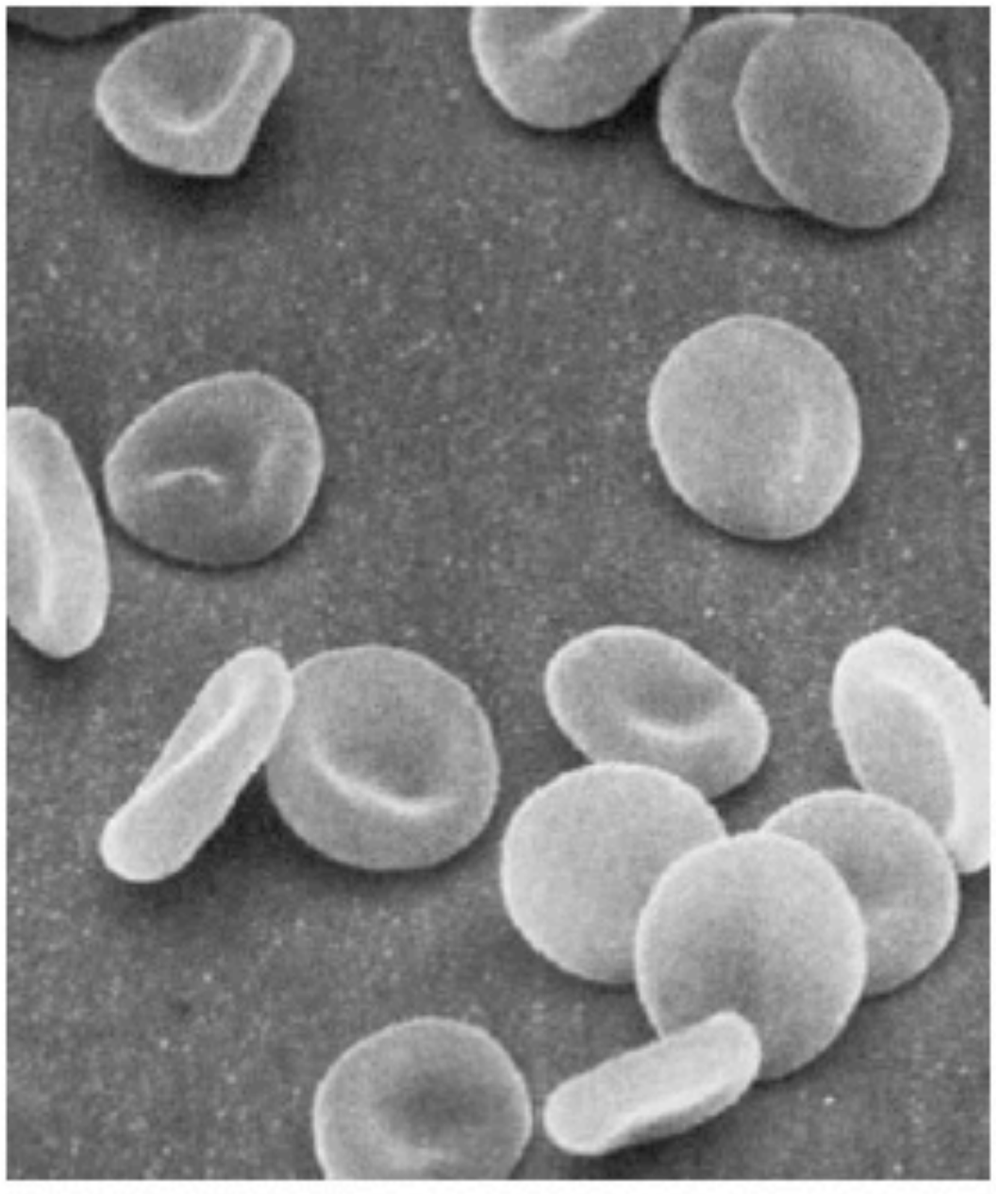
Biological Uses of Cooperativity and Allostery

Hemoglobin: Efficient Ligand Delivery

- Hemoglobin binds O_2 reversibly under different partial pressures

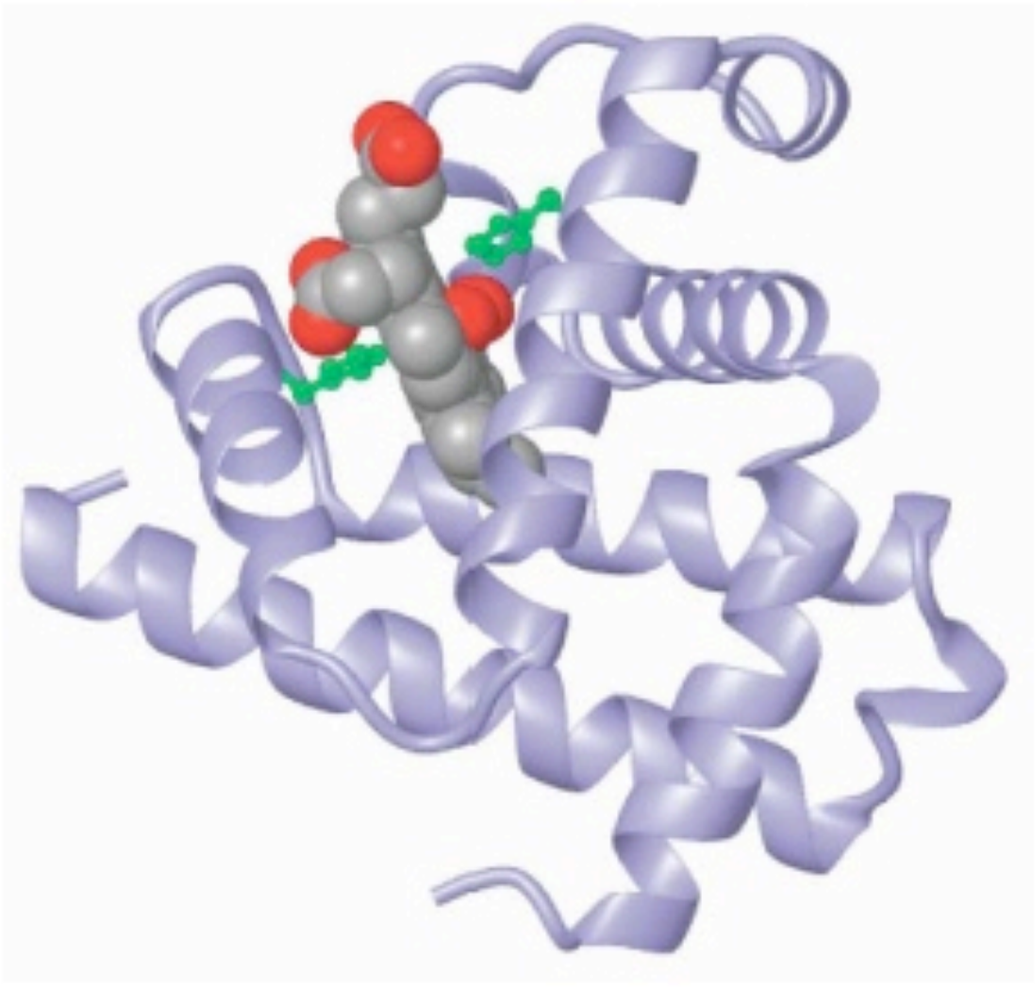
- Why make hemoglobin cooperative?

- 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

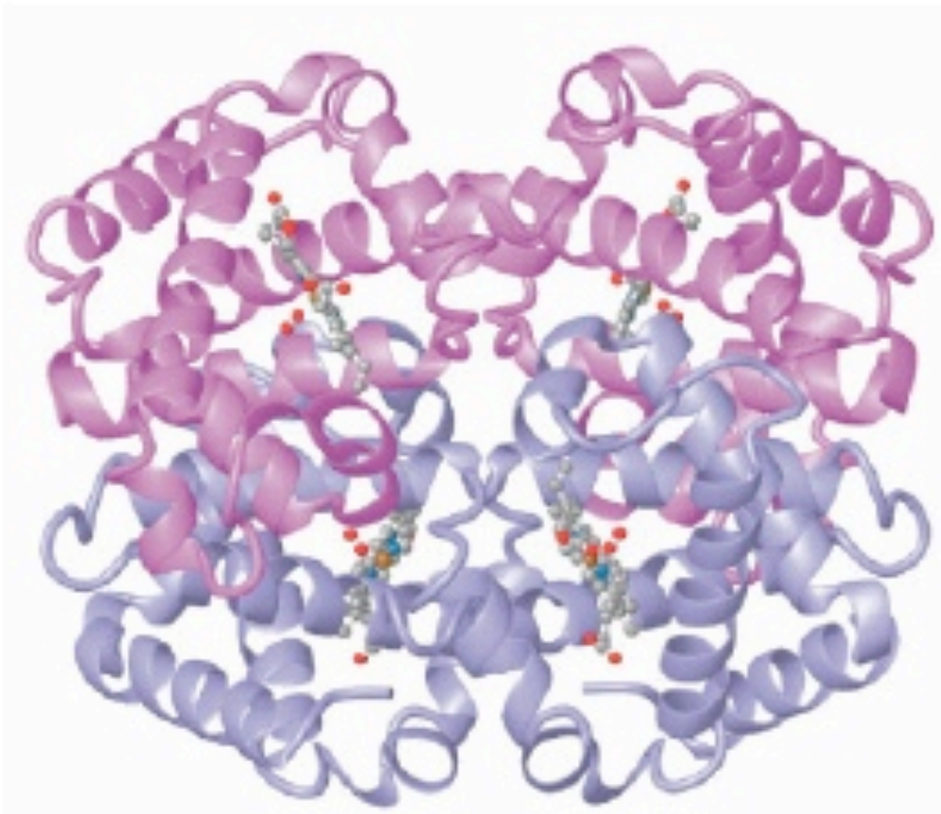


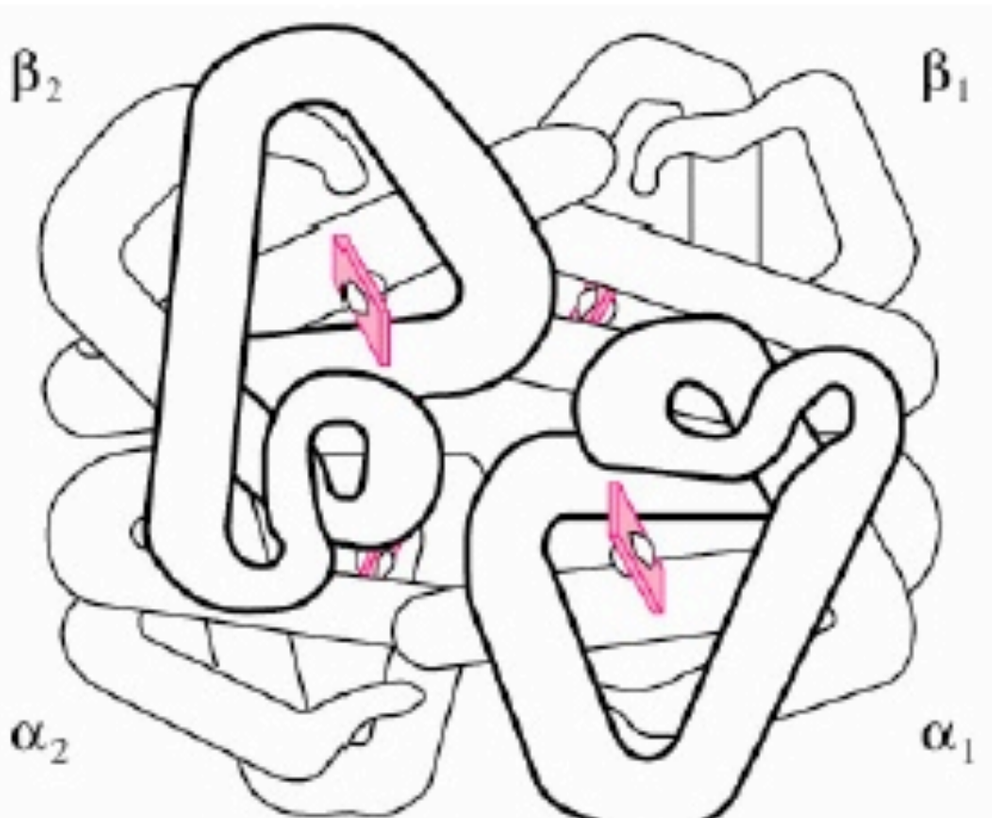
Myoglobin

- 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.

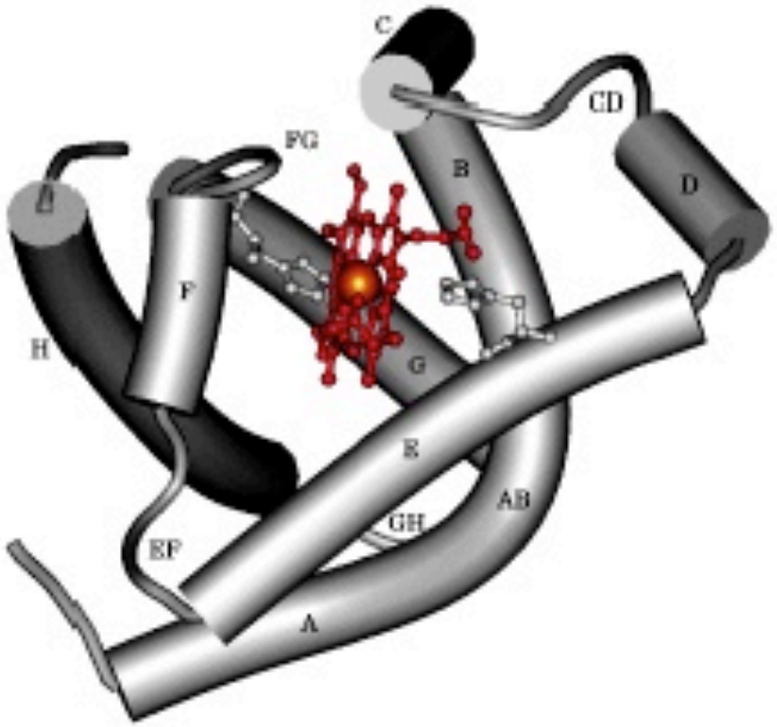
Hemoglobin

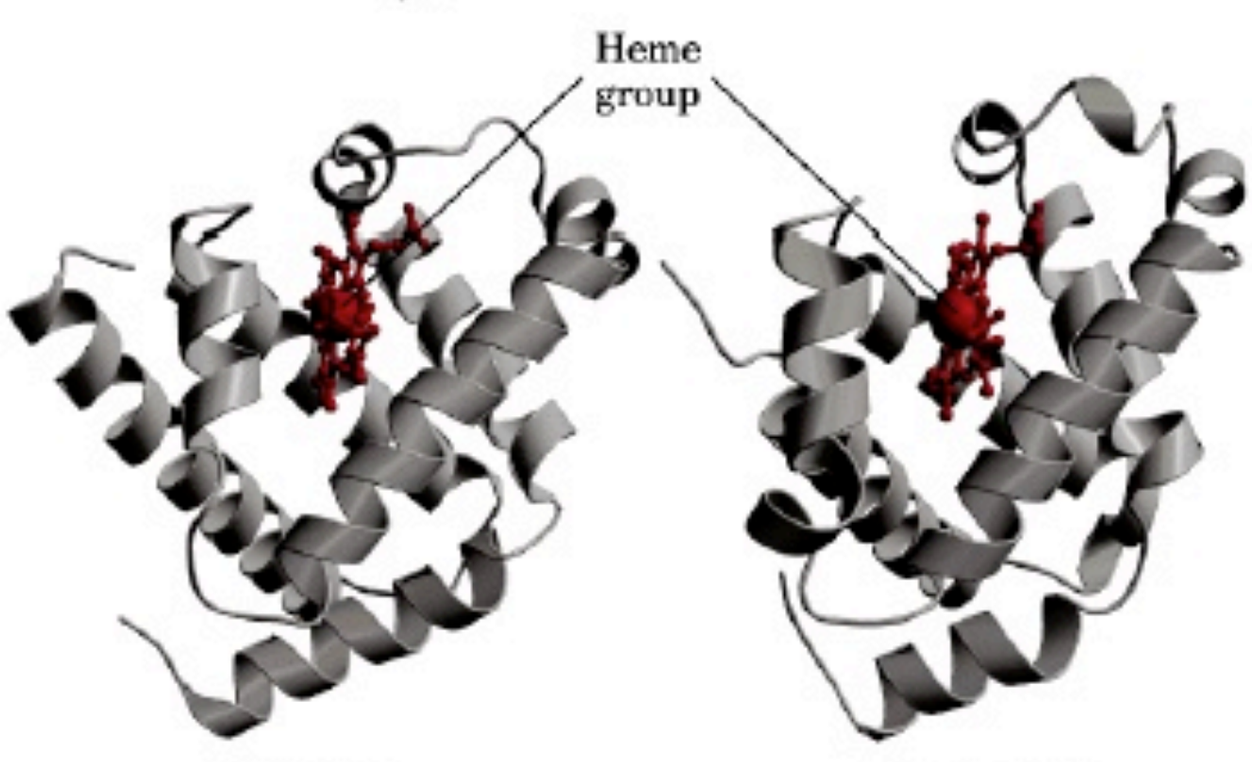
- Tetramer composed of two α-subunits and two β-subunits ($\alpha 2\beta 2$ tetramer).
- The α-subunit is 141 residues and the β-subunit is 146 residues.
- Each polypeptide chain is structurally similar to myoglobin.
- Each polypeptide chain contains a covalently bound heme group.





Structural Similarities between Myoglobin and Hemoglobin



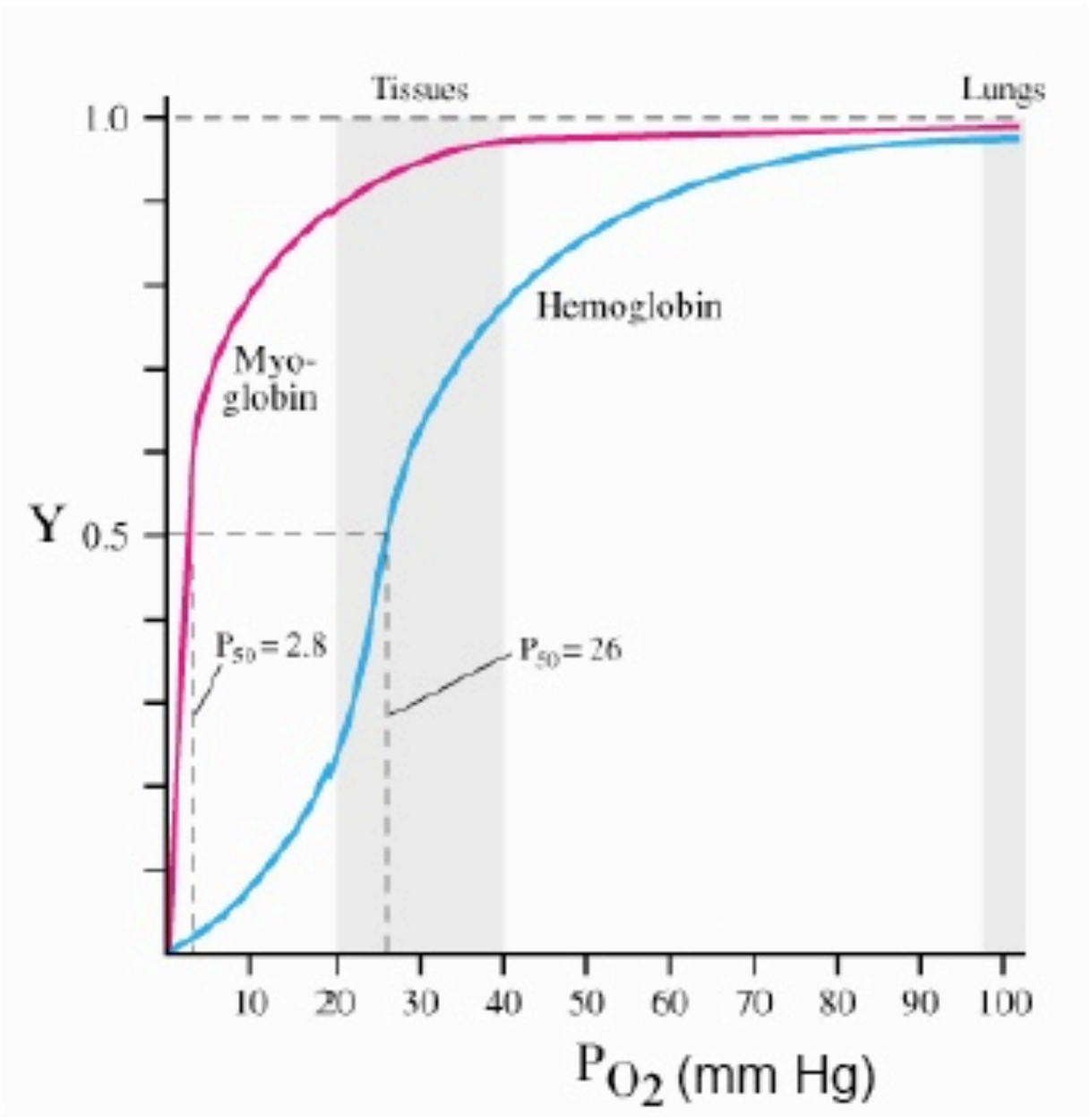


Myoglobin

β subunit of
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.

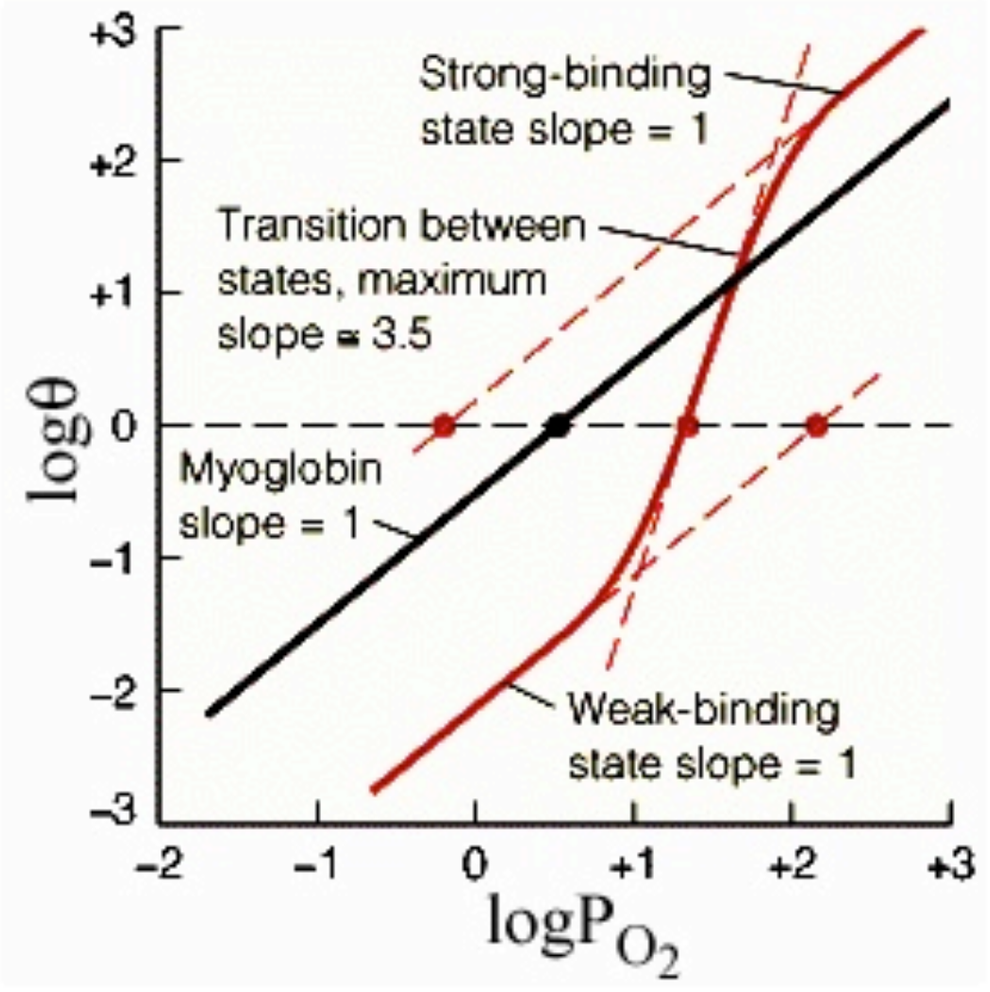


The free oxygen is expressed as the partial pressure of oxygen (P_{O_2}).

Myoglobin vs. Hemoglobin

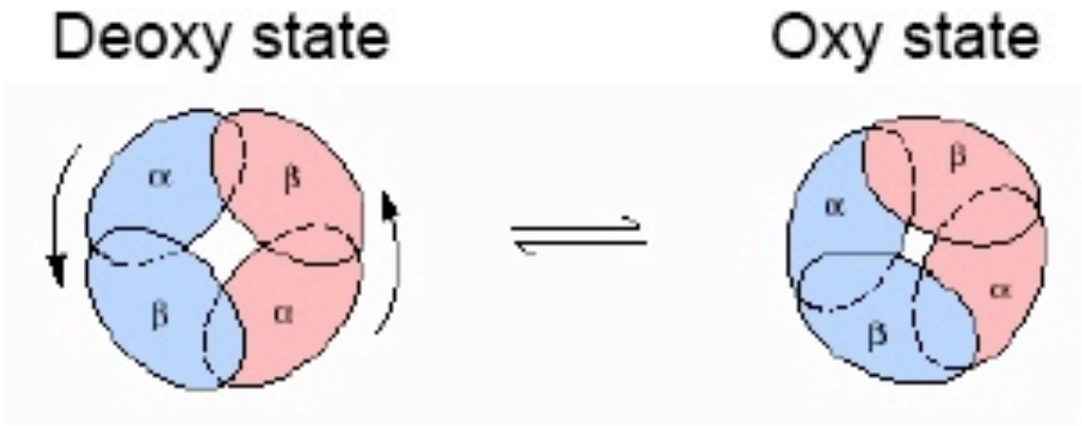
- Hemoglobin binds O_2 less tightly.
- Hemoglobin displays cooperativity (*i.e.* binding of one O_2 molecule increases the affinity for subsequent O_2 binding).
- Hemoglobin saturates at about the same O_2 concentration as myoglobin, but releases essentially all of its O_2 cargo at much higher partial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

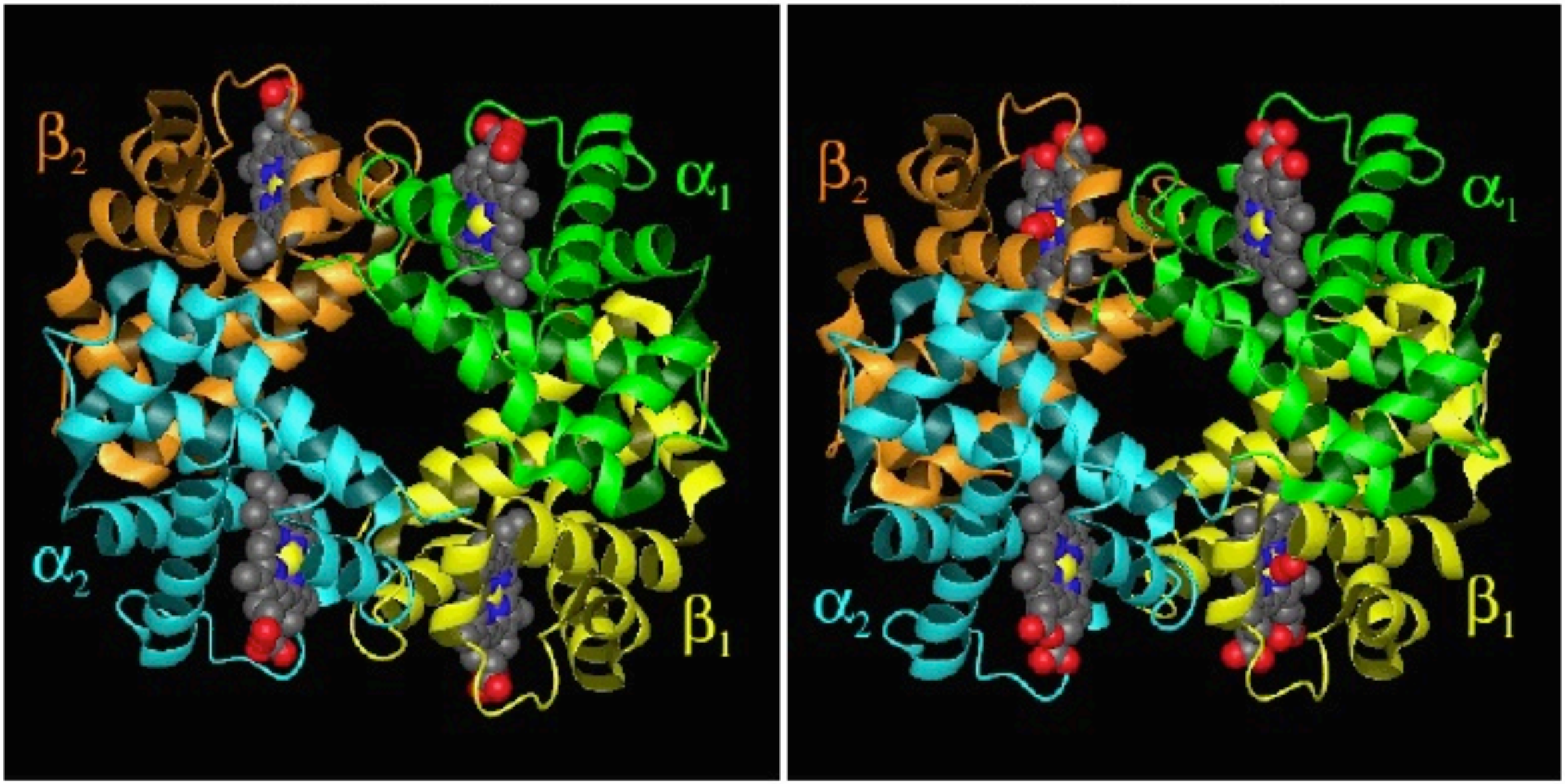


- O_2 binding causes a series of shifts in all subunits, one $\alpha\beta$ pair rotates and slides with respect to the other pair.
- There is a change in the heme structure upon binding O_2.
- Since His F8 is covalently attached to the heme, all of helix F shifts.
- The reorganization of helix F alters 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.

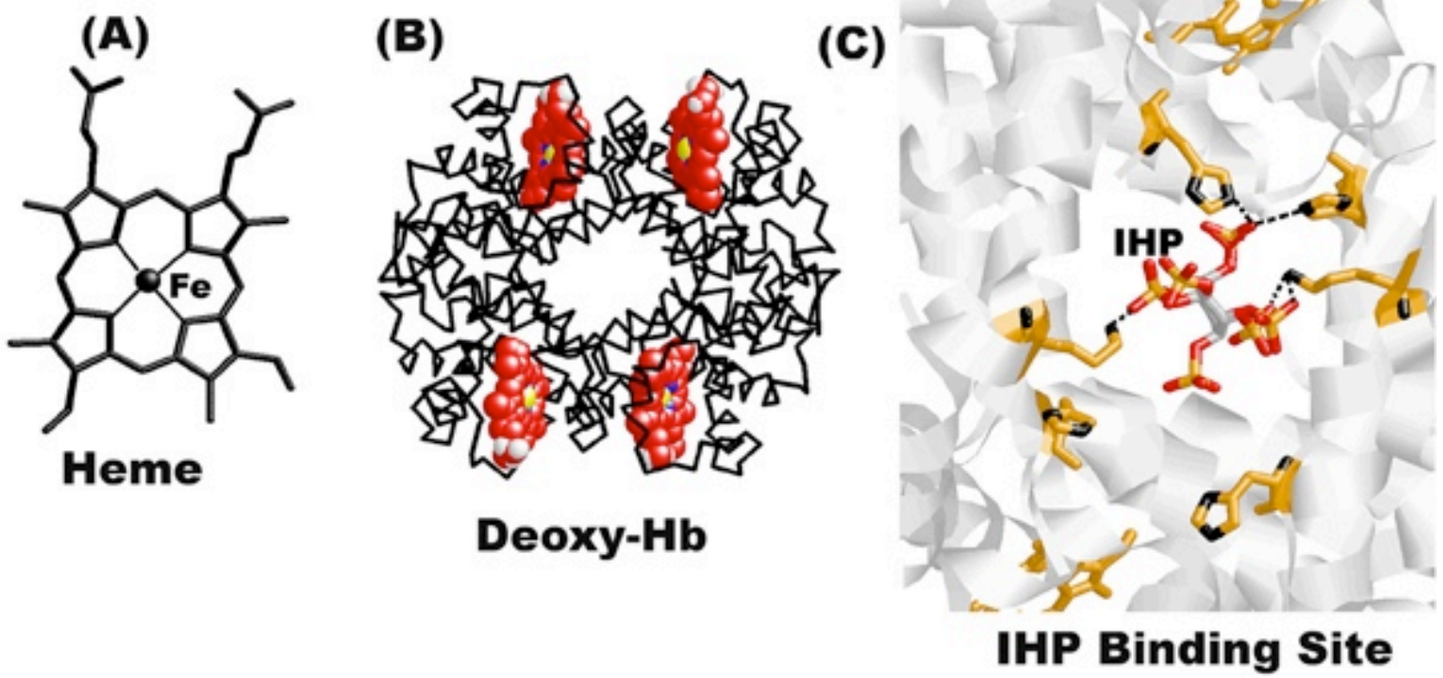
Deoxy-T (tense) state

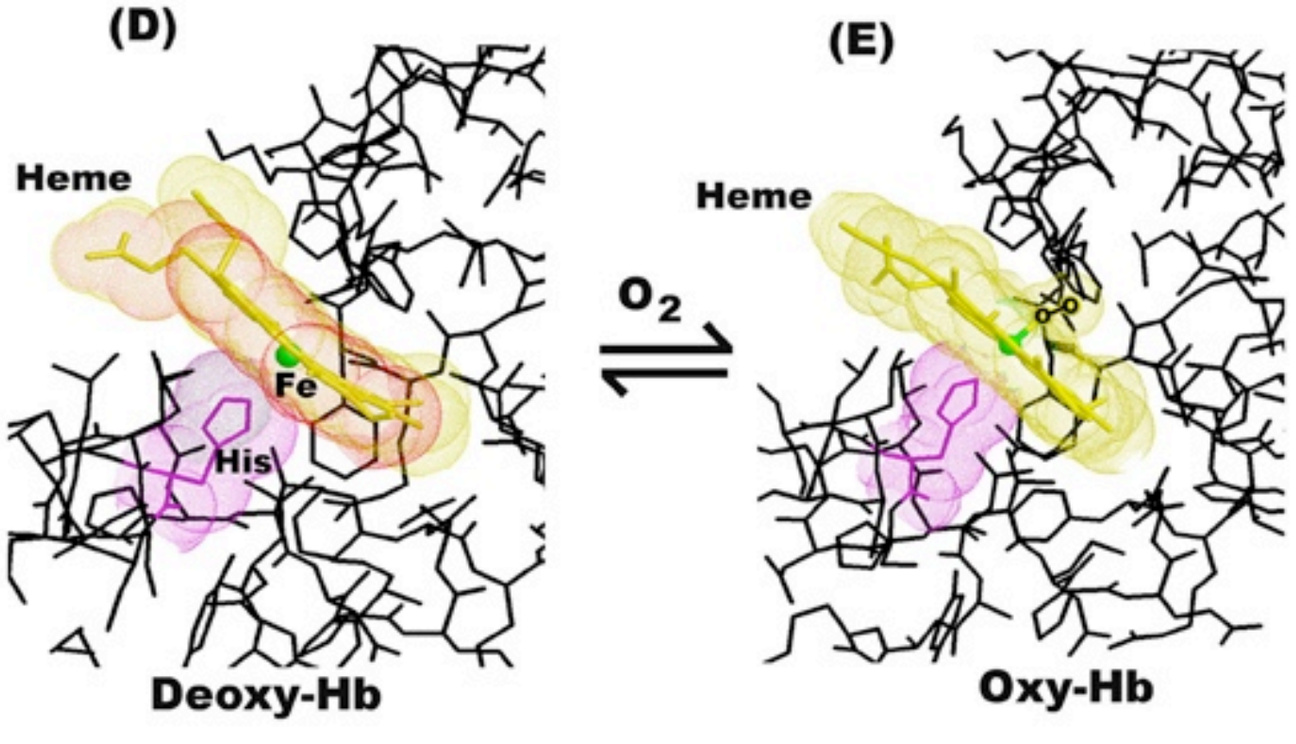
Oxy- R (relaxed) state



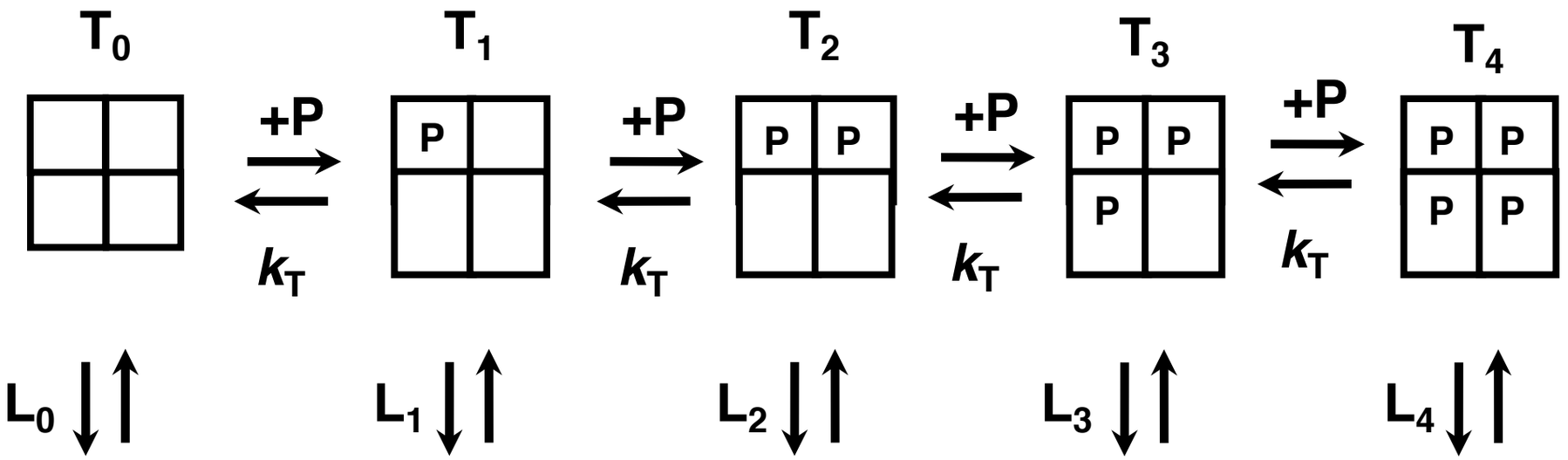
Hemoglobin Gallery of still pictures and animations by Dr. John Lukin
http://www.andrew.cmu.edu/user/jl2p/Hb_html/gallery.html

Binding of dioxygen to hemoglobin

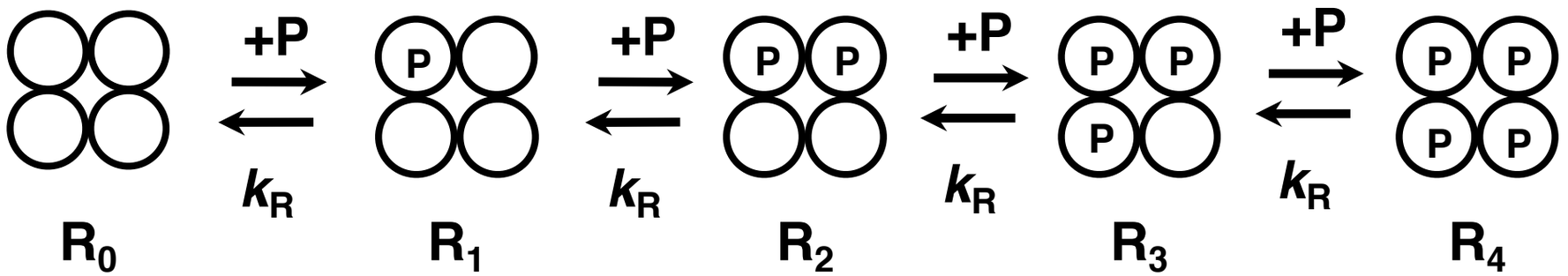




The Monod-Wyman-Changeau (MWC) model for cooperative binding



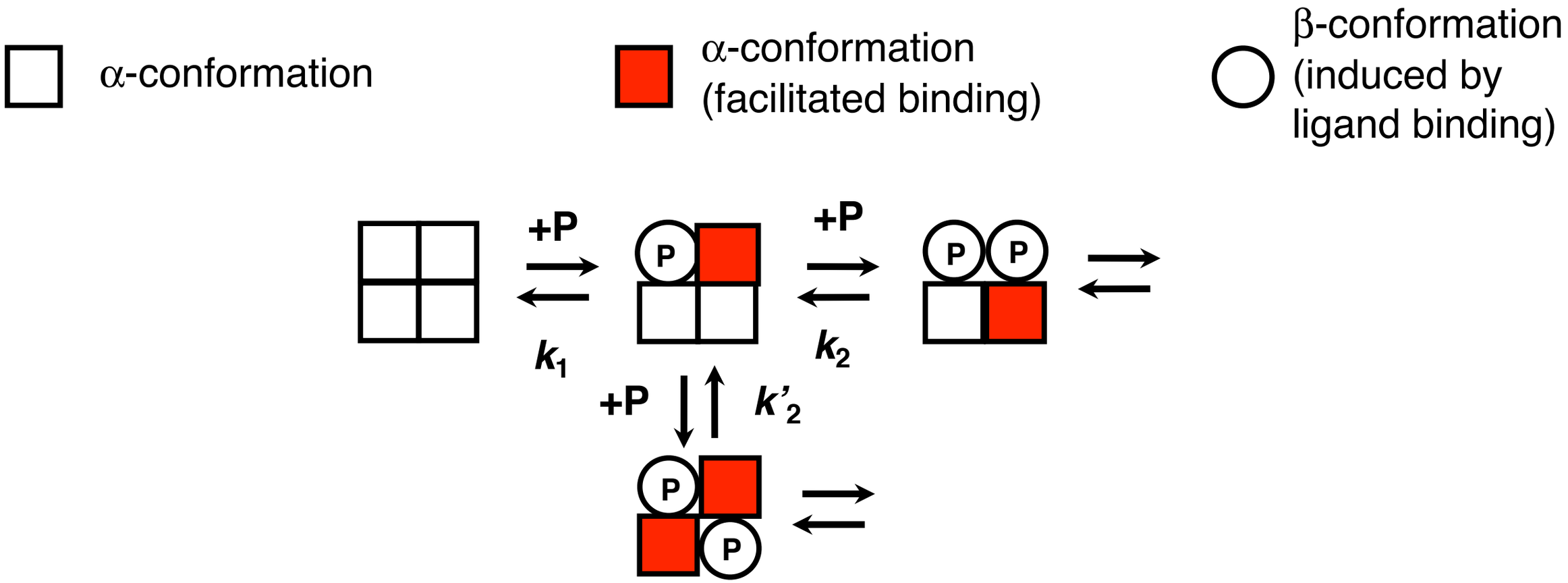
T conformation (all binding sites are weak)



R conformation (all binding sites are strong)

- **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**

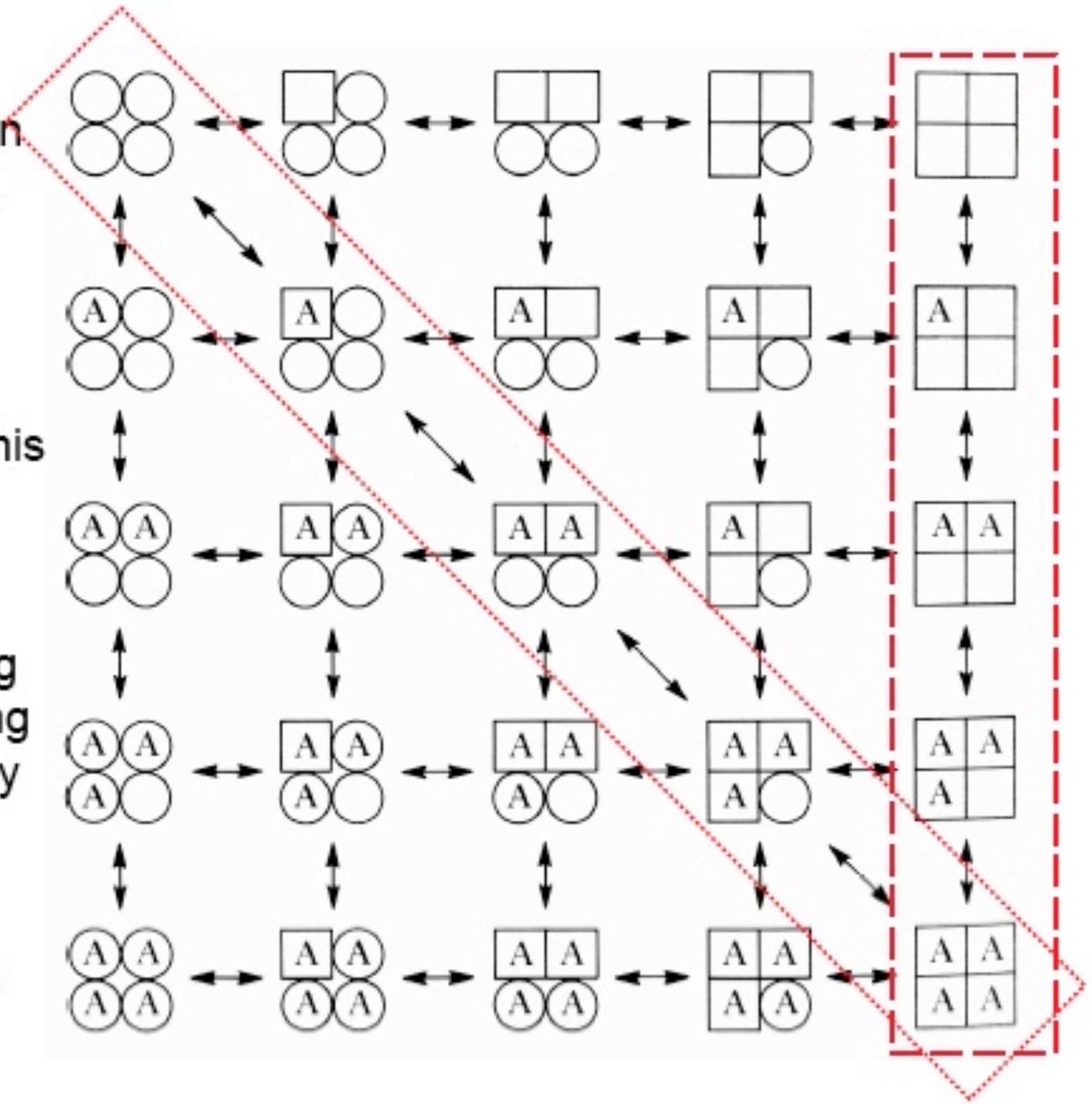
The Koshland-Nemethy-Filmer (KNF) model for cooperative binding



- **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.