Increasing complexity of binding

all binding sites are
equivalent and independent

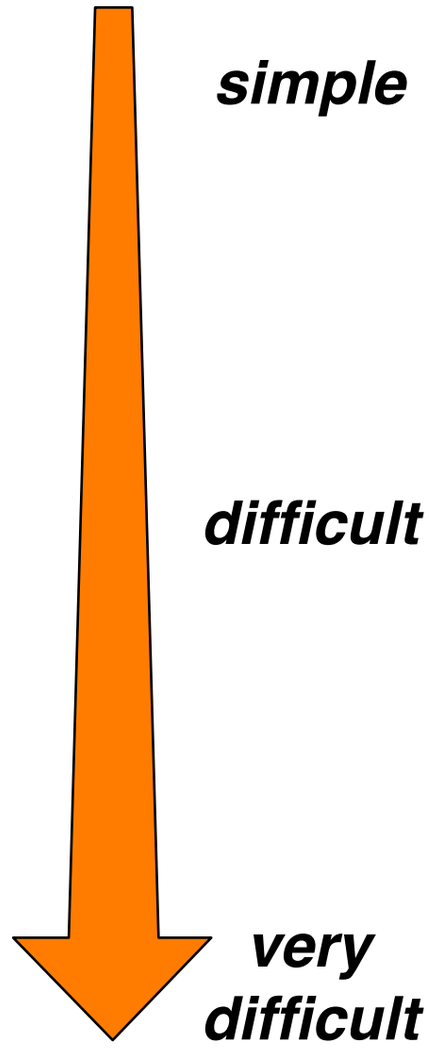
cooperativity ↙ ↘ ***heterogeneity***

all binding sites are
equivalent and not independent

all binding sites are
independent but not equivalent

heterogeneity ↘ ↙ ***cooperativity***

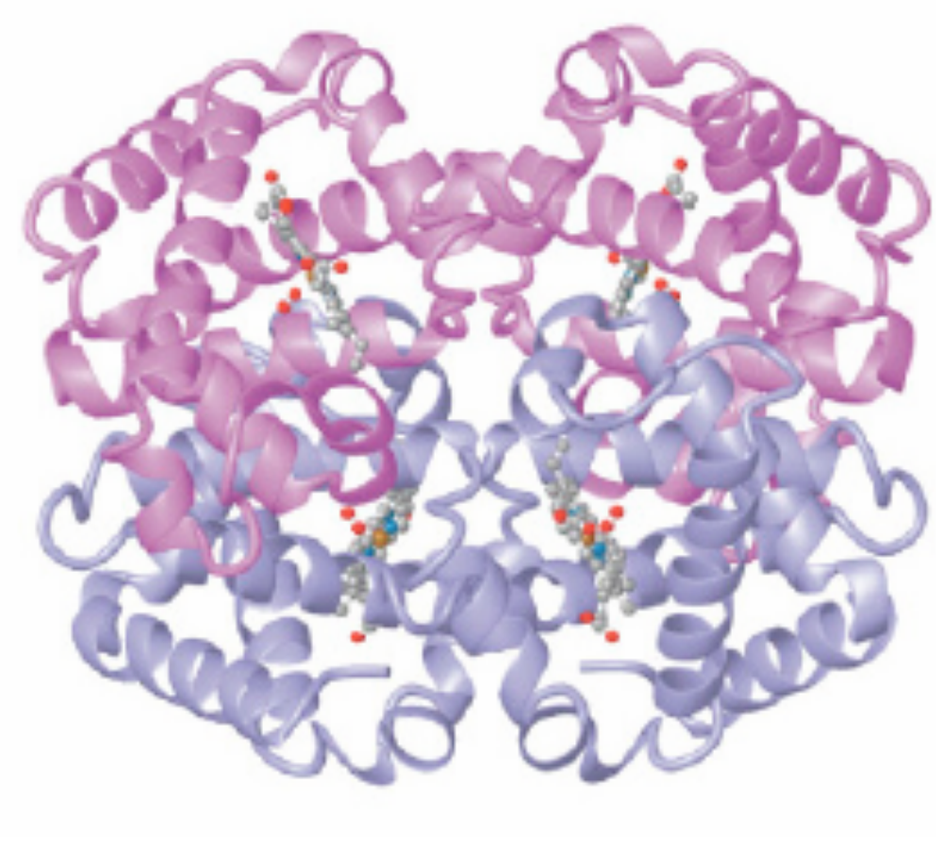
all binding sites are
not equivalent and not independent

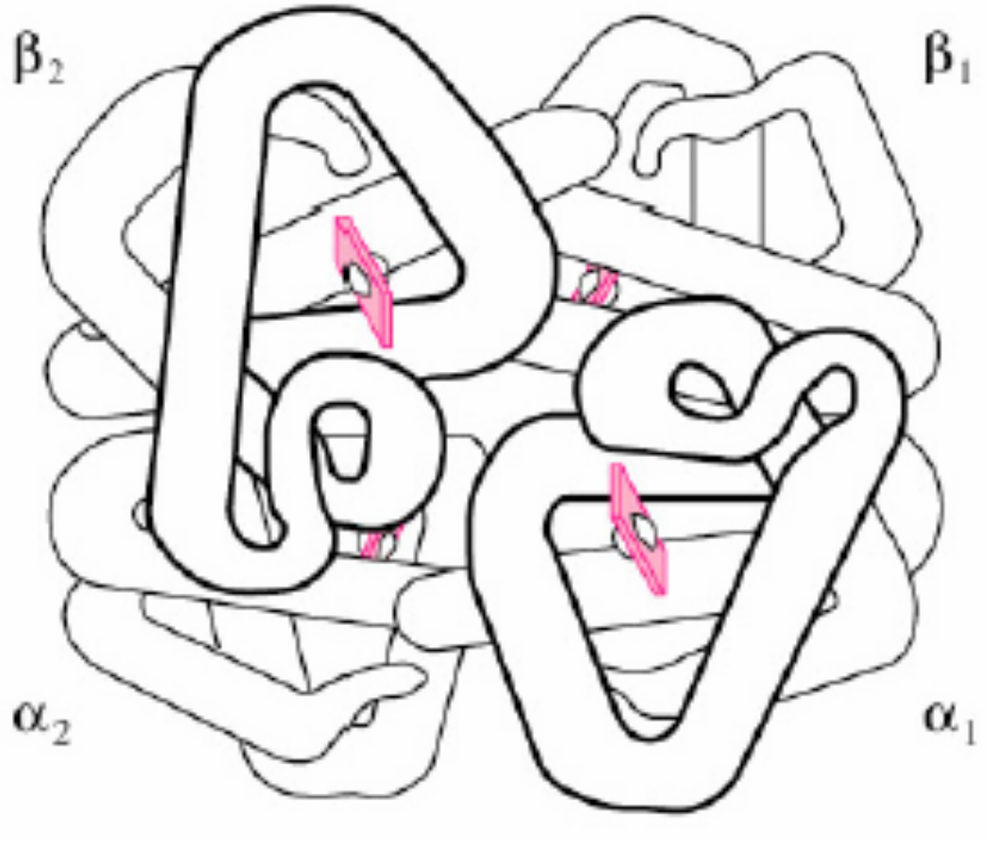


An example for complex binding: oxygen binding to hemoglobin

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.





Biological Uses of Cooperativity and Allostery

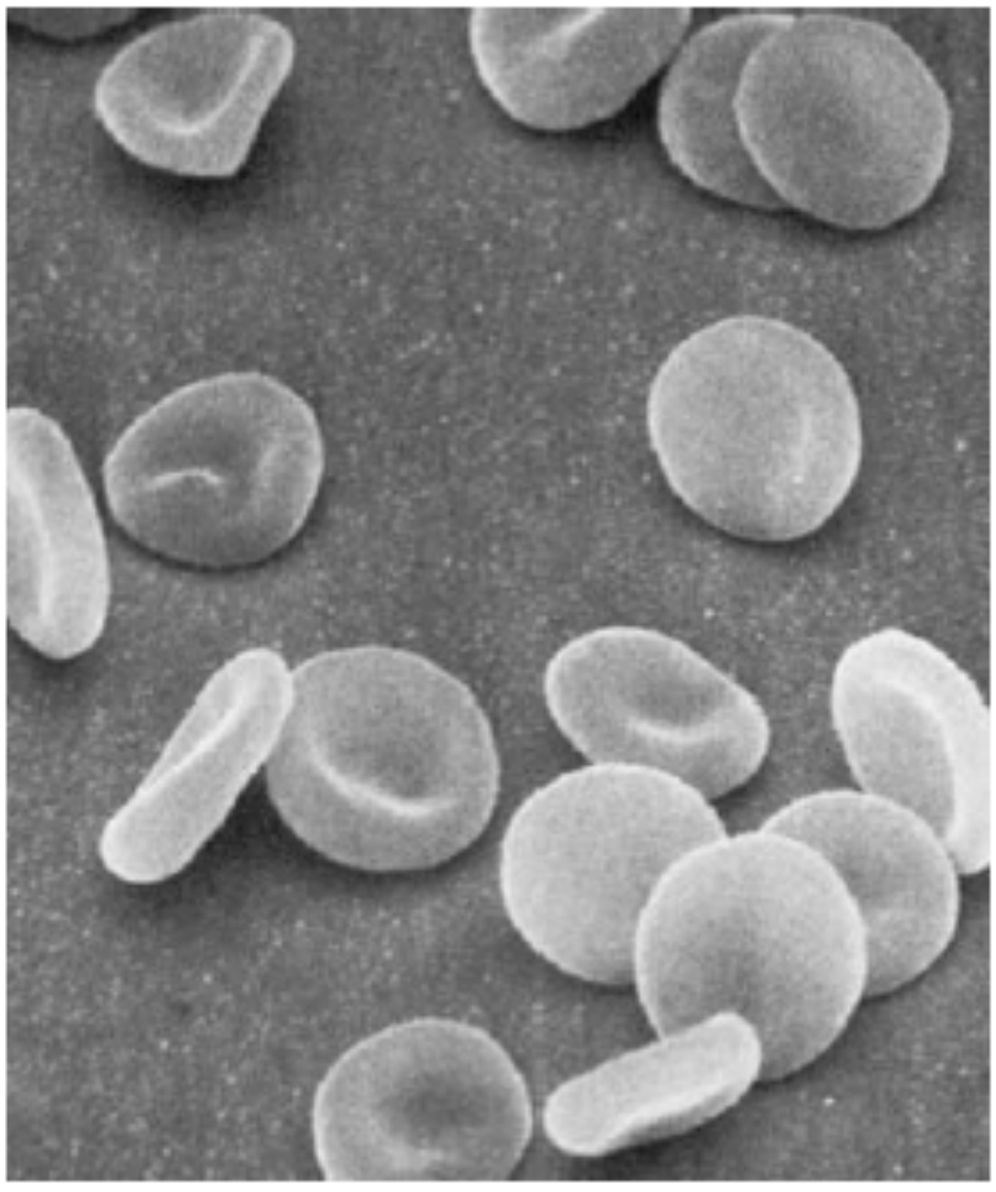
Hemoglobin: Efficient Ligand Delivery

- Hemoglobin binds O_2 reversibly under different partial pressures

- Why make hemoglobin cooperative?

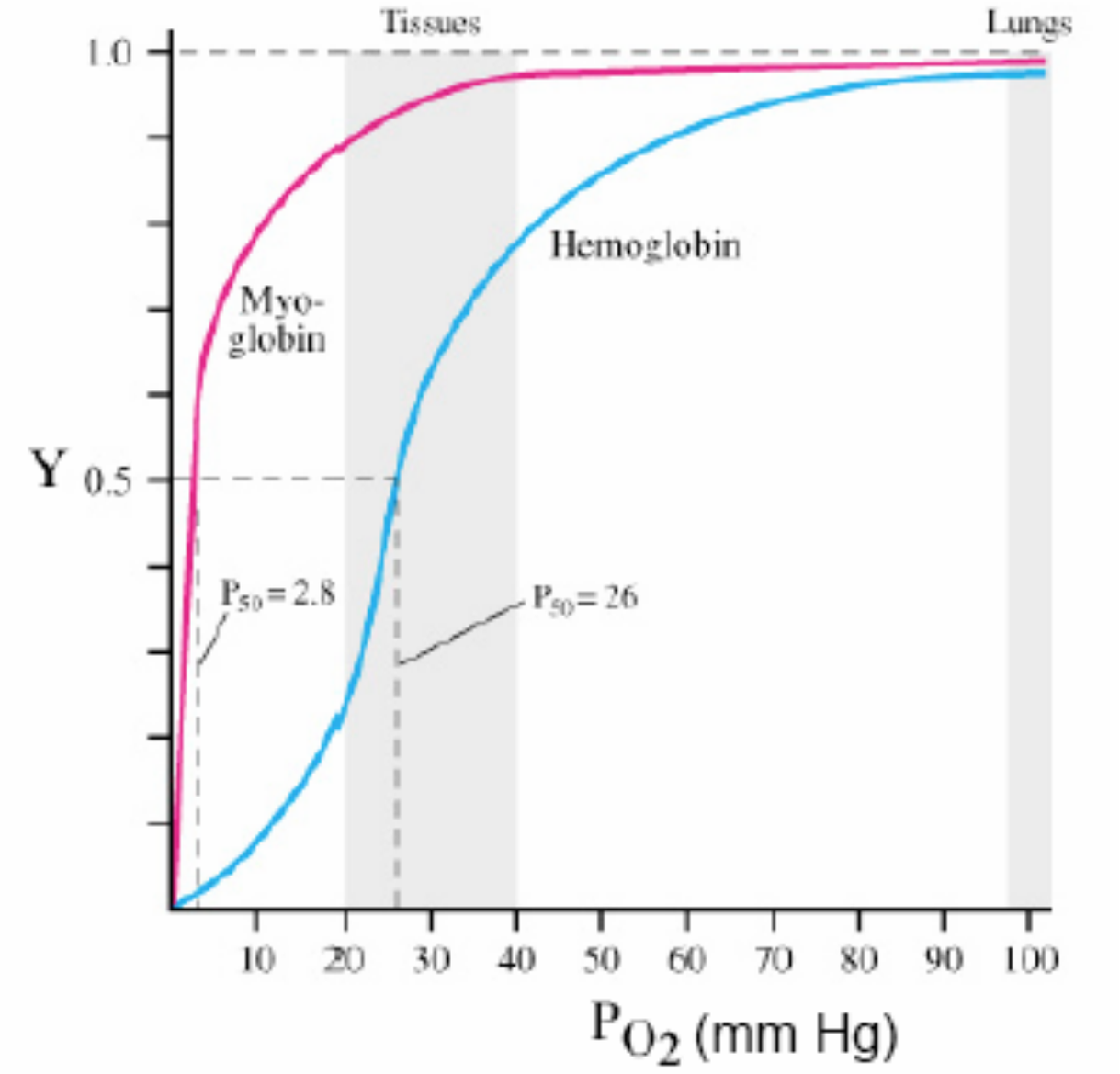
- Positive cooperativity gives all or none behavior. Thus, hemoglobin saturates at about the same O_2 concentration as myoglobin, but releases essentially all of its O_2 cargo at much higher partial pressure of O_2.

Similar scenario for transcriptional regulation: repressor/activator that becomes active/ inactive in the ligand bound state, see Phillips, R. (2015). Napoleon Is in Equilibrium. Annu Rev Condens Matter Phys.



Each erythrocyte contains ~300 million hemoglobin molecules.

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.

How to describe the ligand binding curve to a macromolecule with 4 binding sites?

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

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

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

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

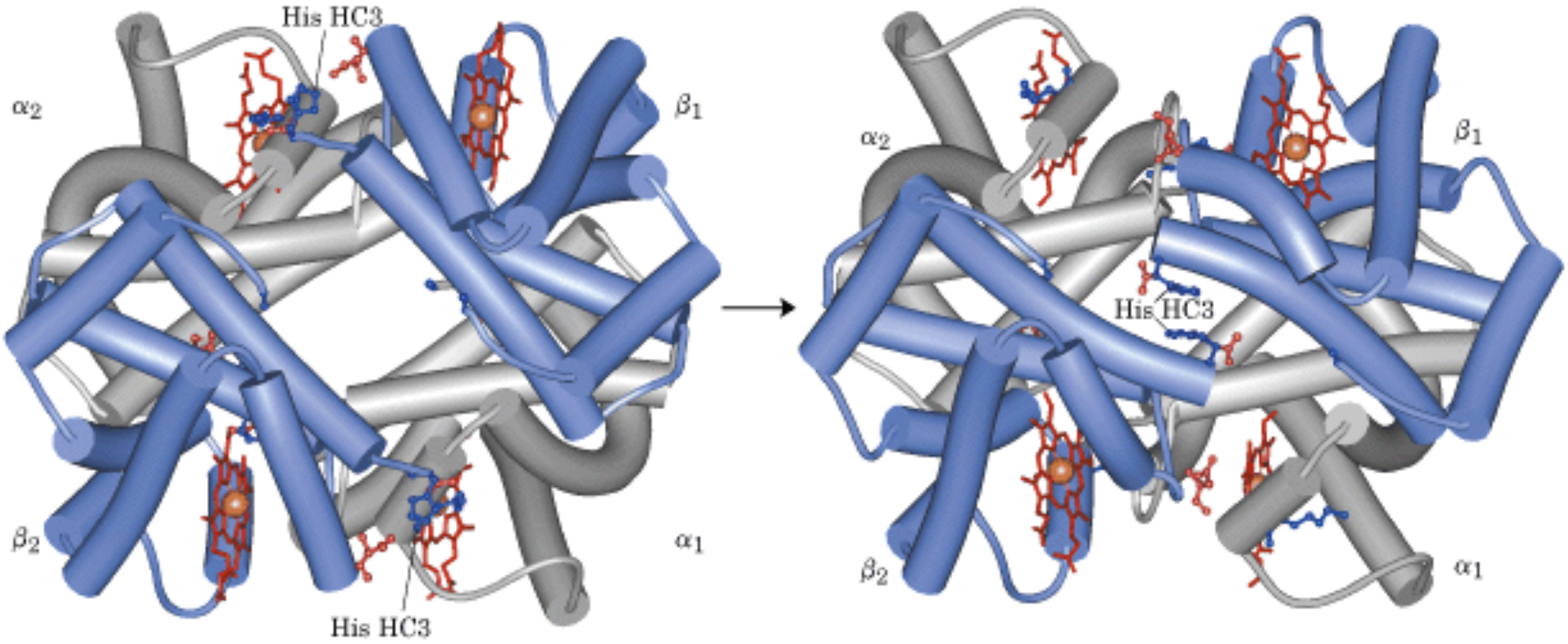
$$\nu_4 = \frac{\frac{1}{K_1} \cdot P_{\text{free}}^1 + \frac{2}{K_2} \cdot P_{\text{free}}^2 + \frac{3}{K_3} \cdot P_{\text{free}}^3 + \frac{4}{K_4} \cdot P_{\text{free}}^4}{1 + \frac{1}{K_1} \cdot P_{\text{free}}^1 + \frac{1}{K_2} \cdot P_{\text{free}}^2 + \frac{1}{K_3} \cdot P_{\text{free}}^3 + \frac{1}{K_4}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

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

“Allostery”: Modifying activity by ligand binding induced switching between different conformational states

Deoxy T (tense) state
with low binding affinity

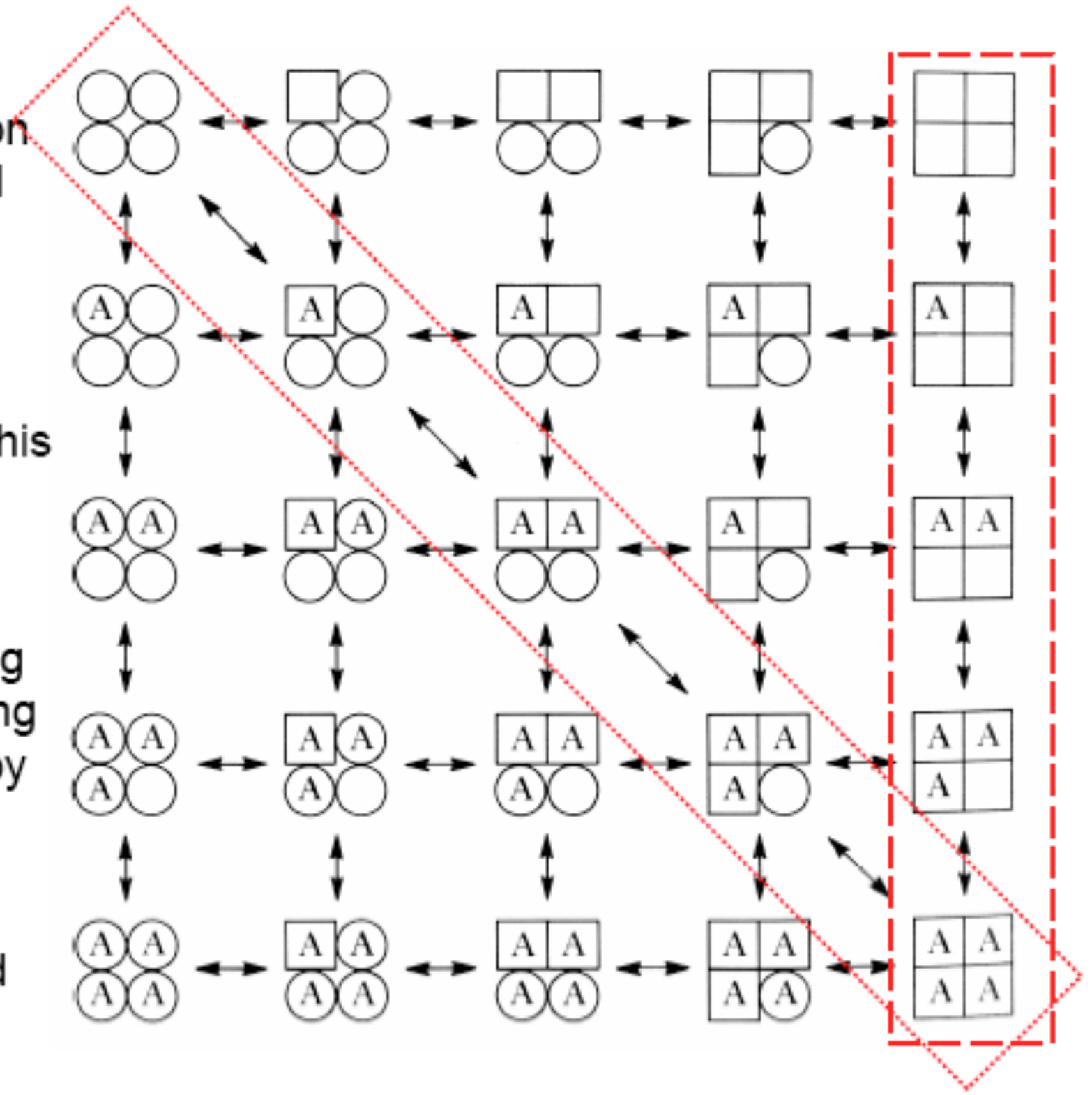
Oxy R (relaxed) state
with high binding affinity



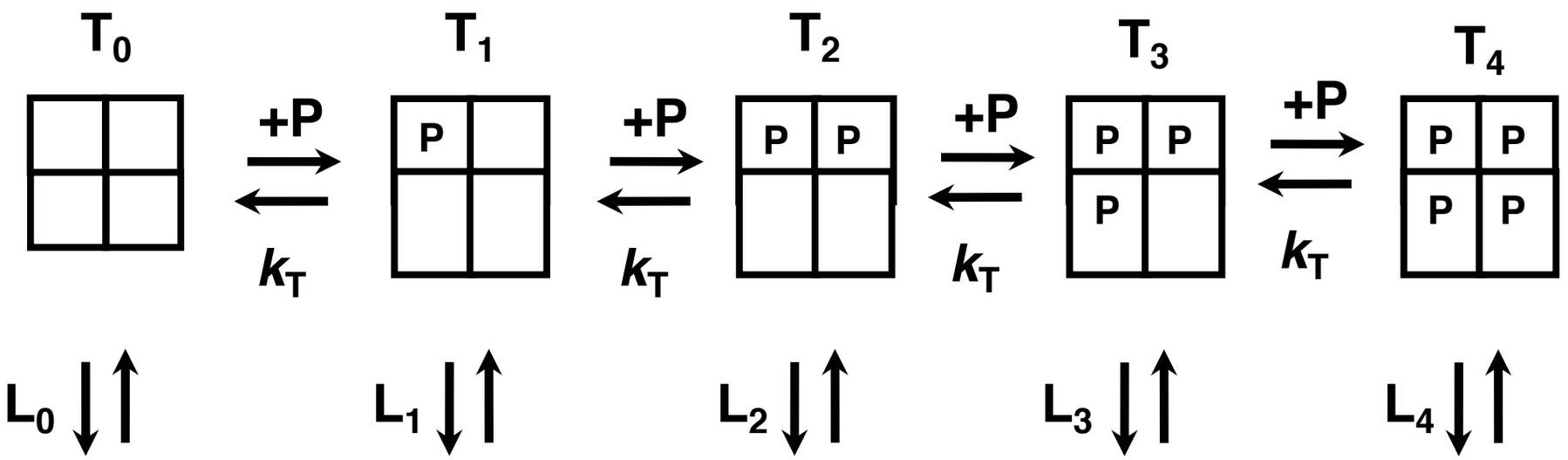
T state **R state**

A more general allosteric scheme…

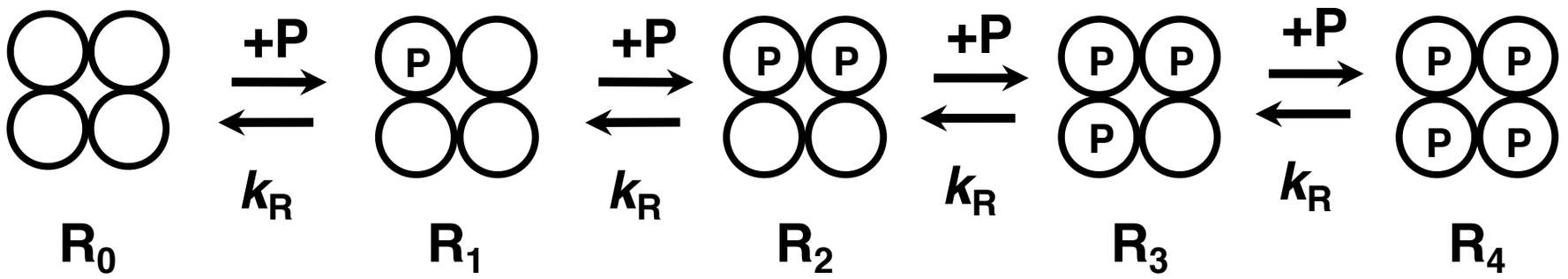
- This scheme allows the individual subunits to take on either of two conformational forms, regardless of the number of ligands that are bound.
- For a four-subunit protein, this allow 25 different combinations.
- The MWC model is a limiting case of this scheme involving only the species enclosed by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



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 Monod-Wyman-Changeau (MWC) model for allosteric transitions

$$\bar{\nu} = \frac{L\,c\,\alpha(1+c\,\alpha)^{n-1} + \alpha(1+\alpha)^{n-1}}{(1+\alpha)^n + L(1+c\,\alpha)^n}$$

fractional occupancy of hemoglobin with ligand

$$\bar{R} = \frac{(1+\alpha)^n}{(1+\alpha)^n + L(1+c\alpha)^n}$$

protein fraction in the R state

$L = [T]_0/[R]_0$

allosteric constant determined by ratio of proteins in the T and R states in the absence of ligand

$c = K_R/K_T$

ratio of binding constants for R and T states

$\alpha = [X]/K_R$

normalized ligand concentration

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

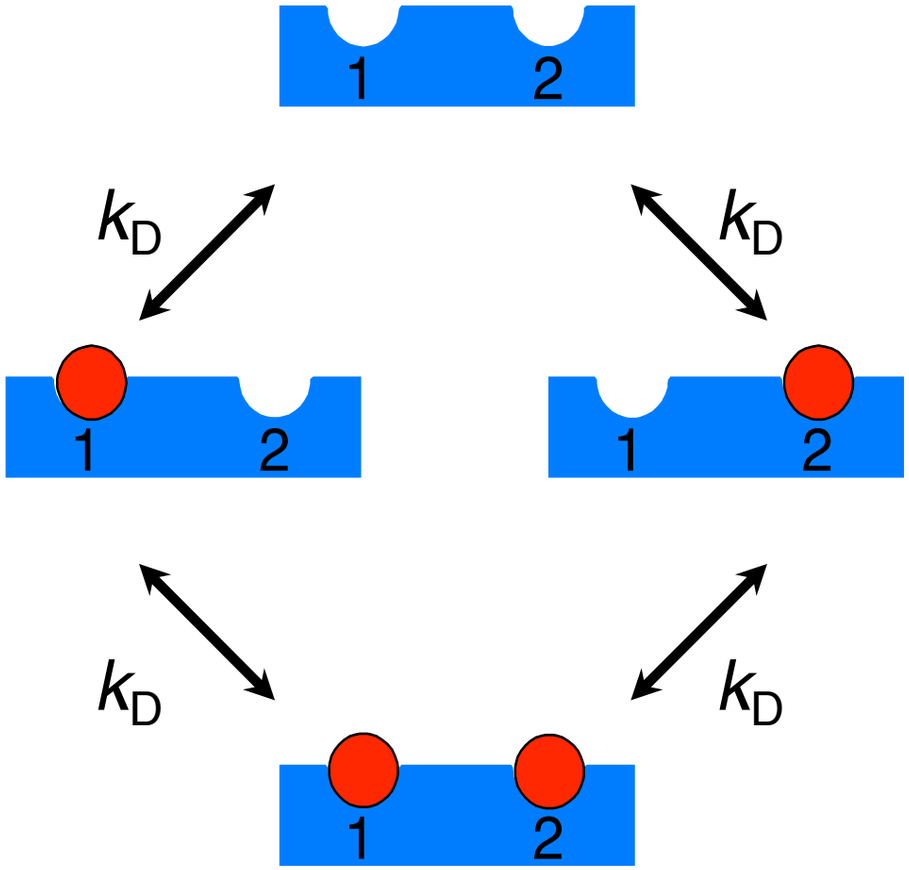
$$D + 2P_{\text{free}} \rightleftharpoons DP_2 \qquad K_2^* = \frac{D_{\text{free}} \cdot P_{\text{free}}^2}{DP_2} \qquad K_2^* = K_1 \cdot K_2$$

alternative expression

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

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

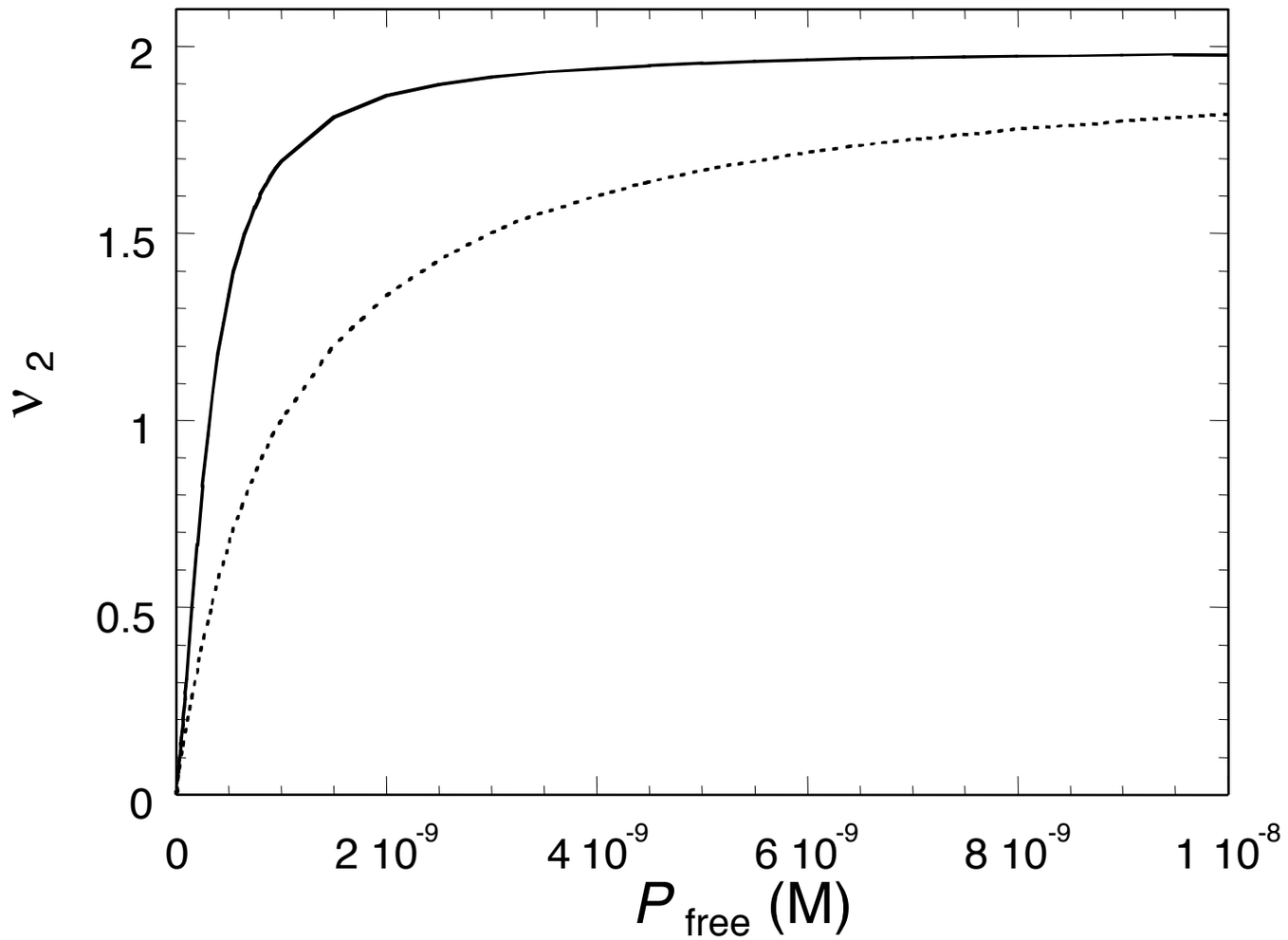
2 possibilities for the dissociation of DP_2

$$K_2 = 2k_d$$

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)

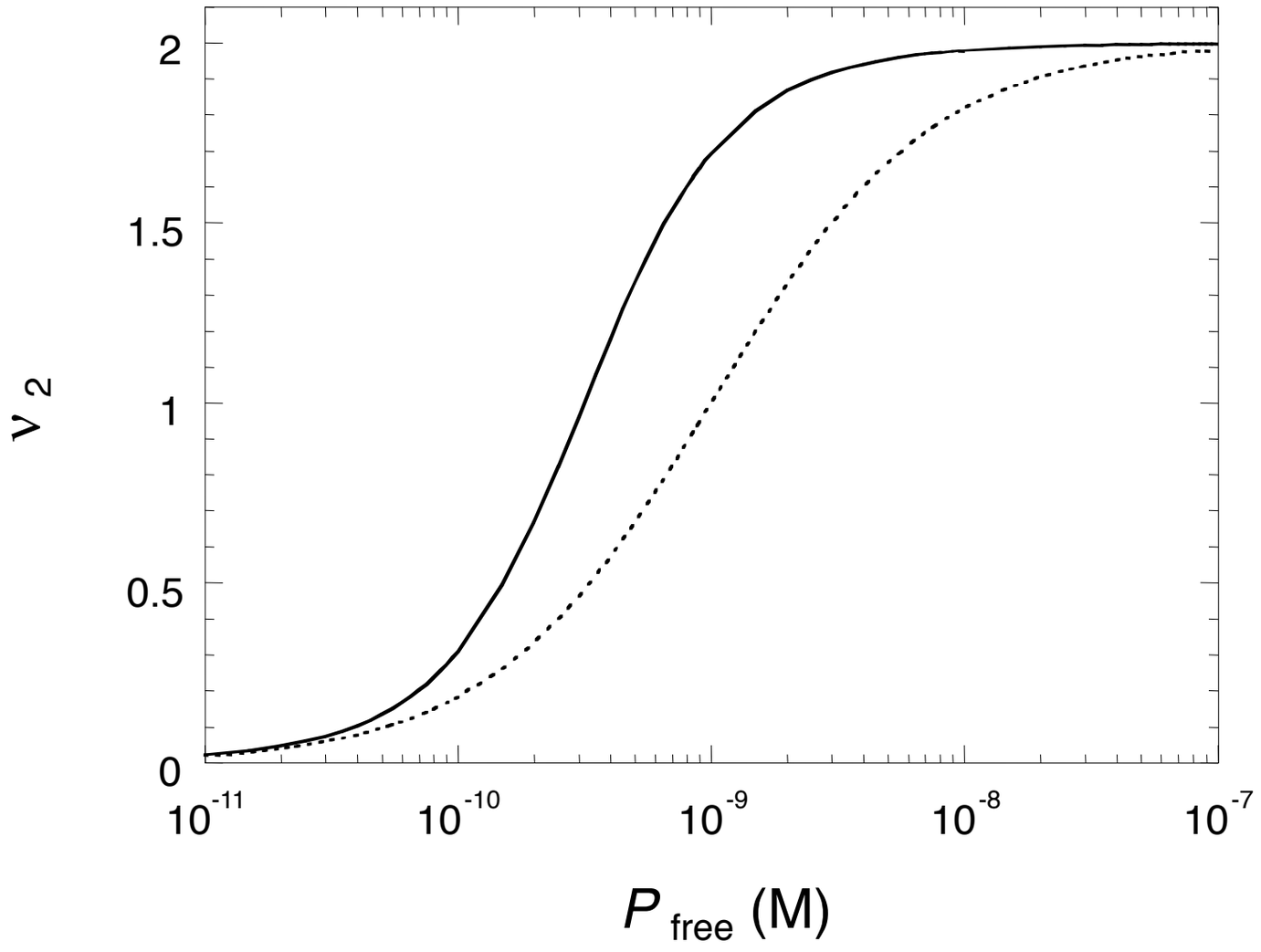
cooperative binding
microscopic binding constant
$k_D = 10^{-9}$ (M)

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

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

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

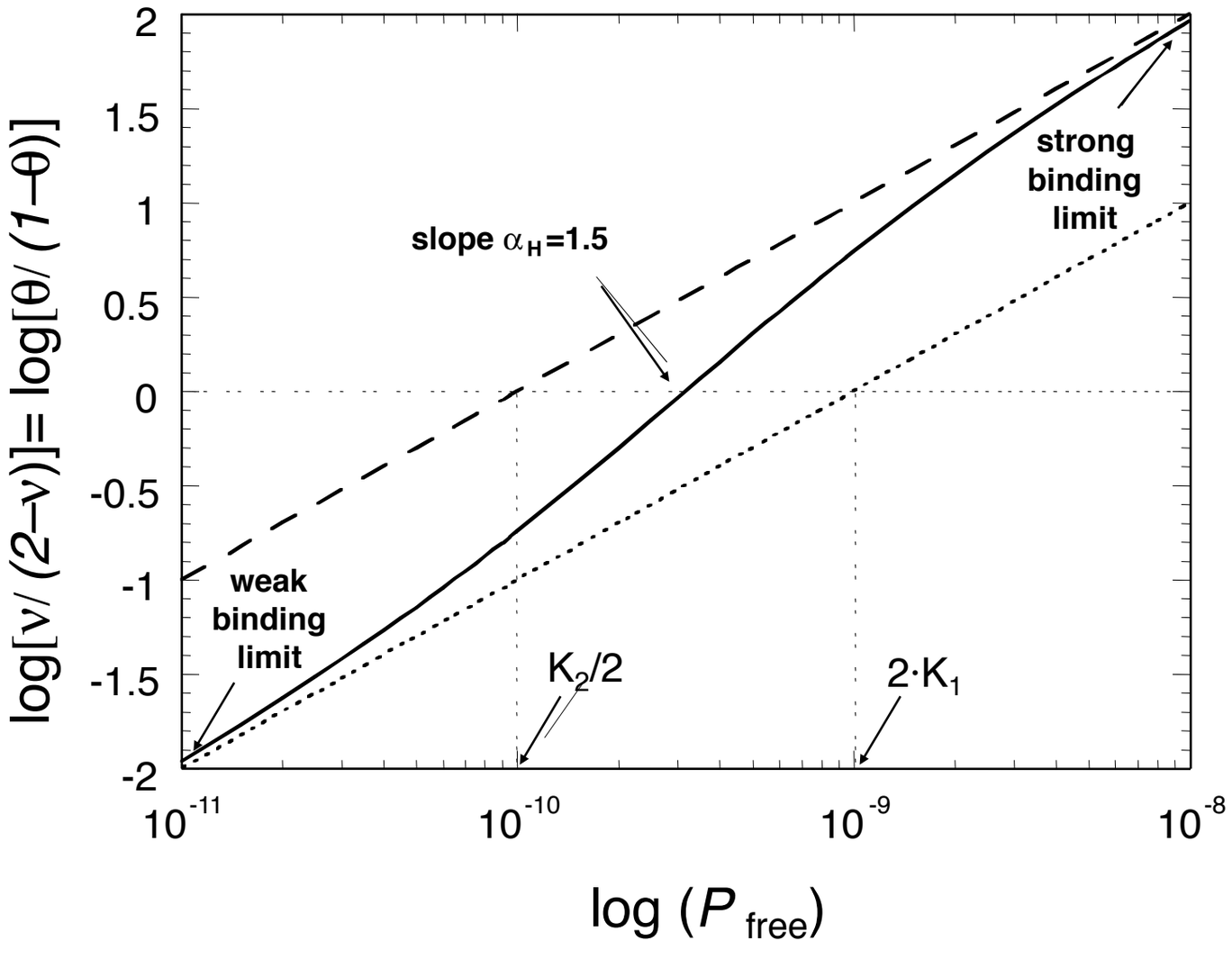
which yields the Hill equation

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

Visualization of binding data - Hill plot

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

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

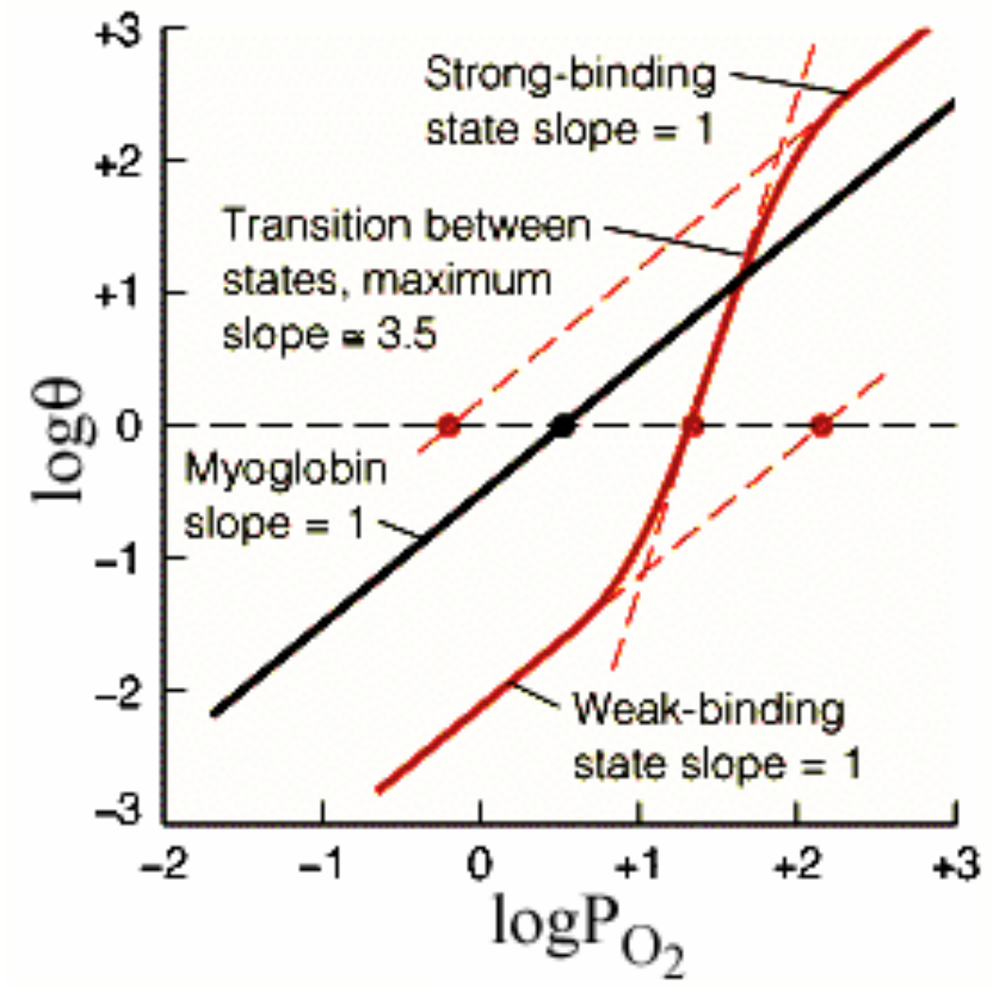


$$P_{free} \to \infty \Rightarrow \log\left(\frac{\nu_2}{2-\nu_2}\right) = \log\left(P_{free}\right) - \log\left(\frac{K_2}{2}\right)$$

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

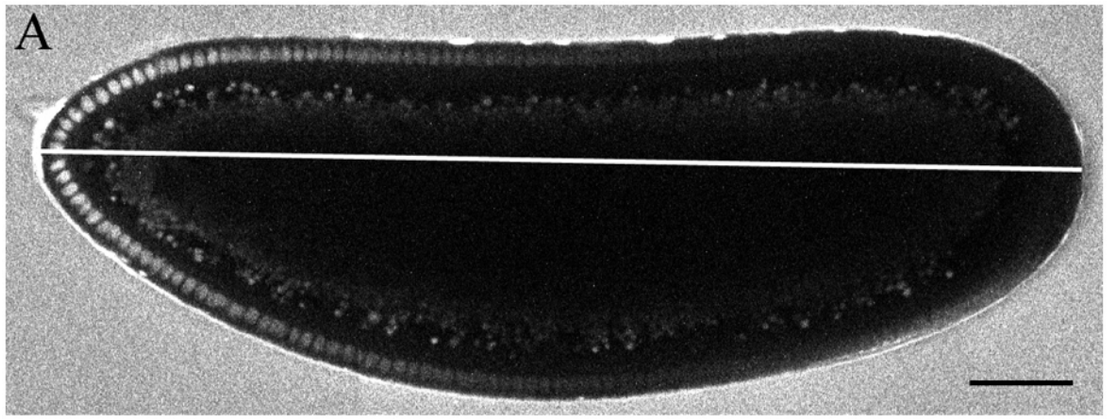
$$P_{free} \to 0 \Rightarrow \log\left(\frac{\nu_2}{2-\nu_2}\right) = \log\left(P_{free}\right) - \log\left(2K_1\right)$$

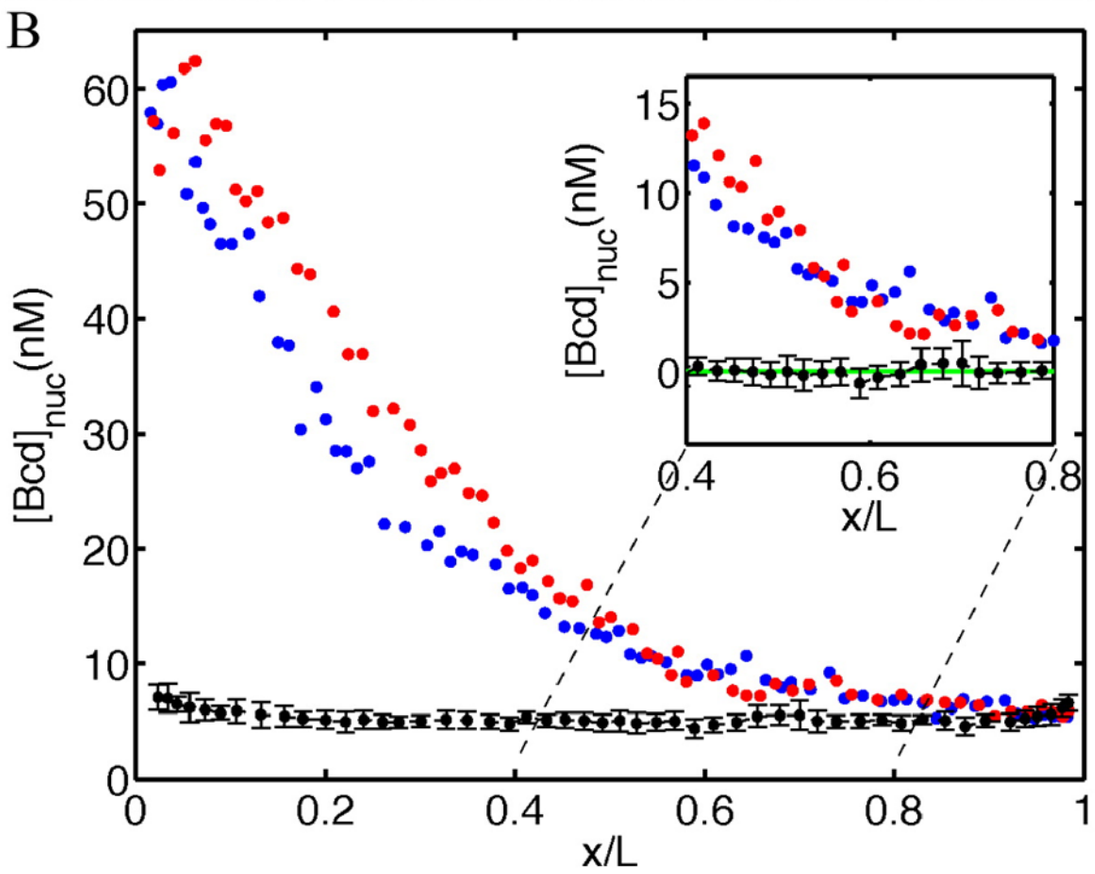
Hill Plots for Oxygen Binding to Hemoglobin and Myoglobin

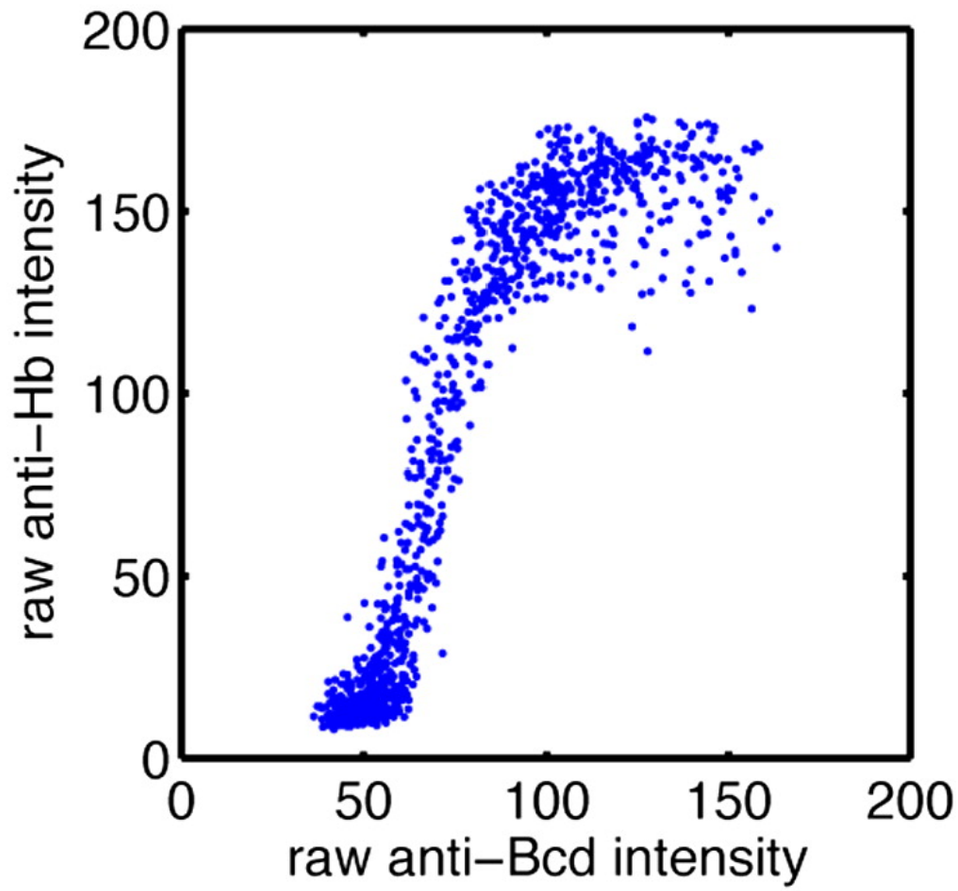


- At low P_{O_2} , the Hill plot has a slope = 1 and corresponds to the weak binding state (large P_{50})
- As binding progresses, the curve switches over to approach another parallel straight line that describes the strong binding state (small P_{50}).
- The transition between binding states is clear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

A gradient of the Bicoid transcription factor in the Drosophila embryo leads to expression of the Hunchback protein

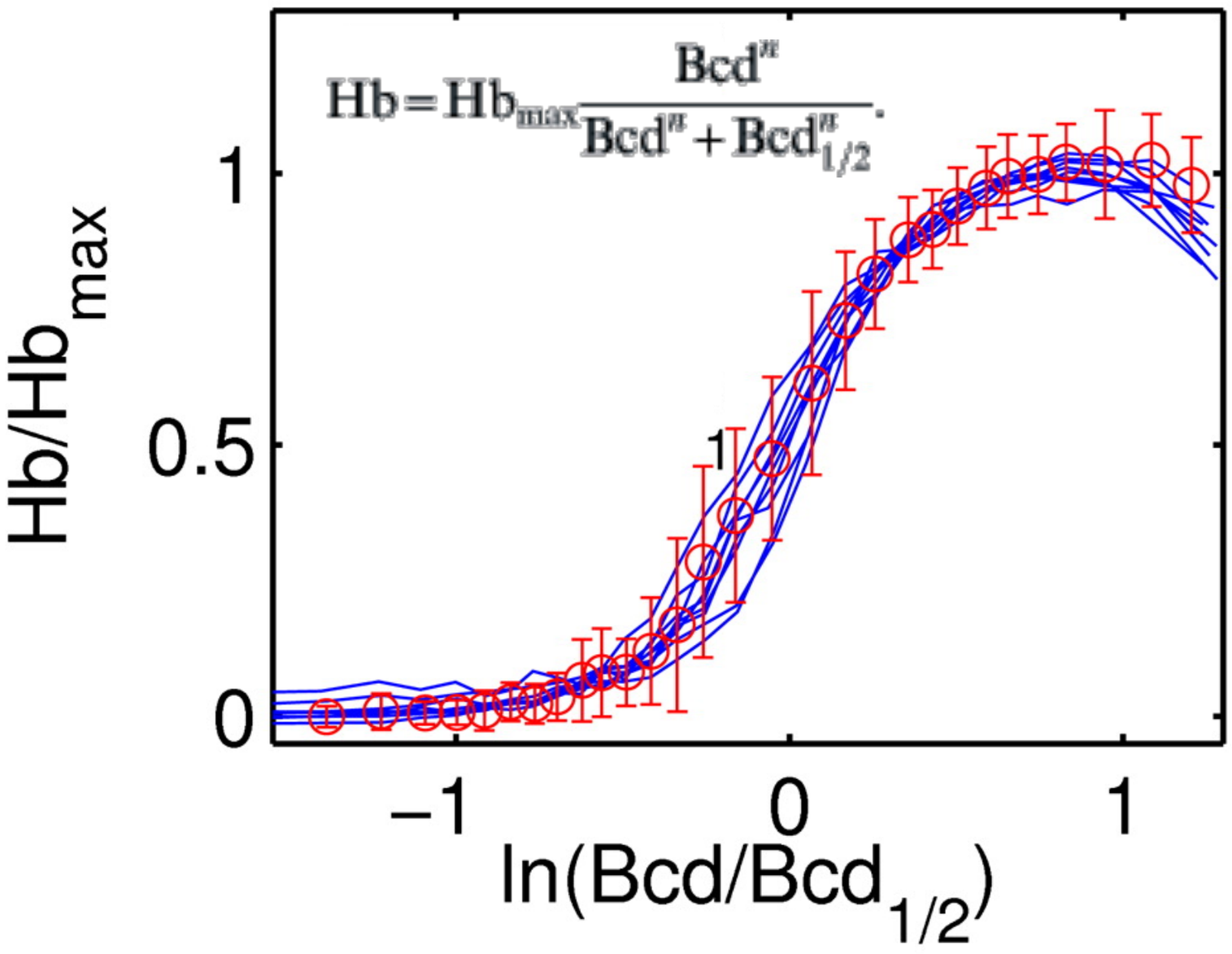






Gregor, T., Tank, D.W., Wieschaus, E.F., and Bialek, W. (2007). Probing the limits to positional information. Cell 130, 153–164.

Best fit of Bicoid-Hunchback relation to Hill equation with n=5



Hb transcription is activated by cooperative binding of Bcd molecules for which 7 Bcd-binding sites are present at the *hb* promoter

Gregor, T., Tank, D.W., Wieschaus, E.F., and Bialek, W. (2007). Probing the limits to positional information. Cell 130, 153–164.

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: $\mathrm{Log}(\theta/(1-\theta))/\mathrm{Log} = f(P_{free})$, $\theta = \nu/n$