# Increasing complexity of equilibrium binding descriptions

all binding sites are
**equivalent and independent**

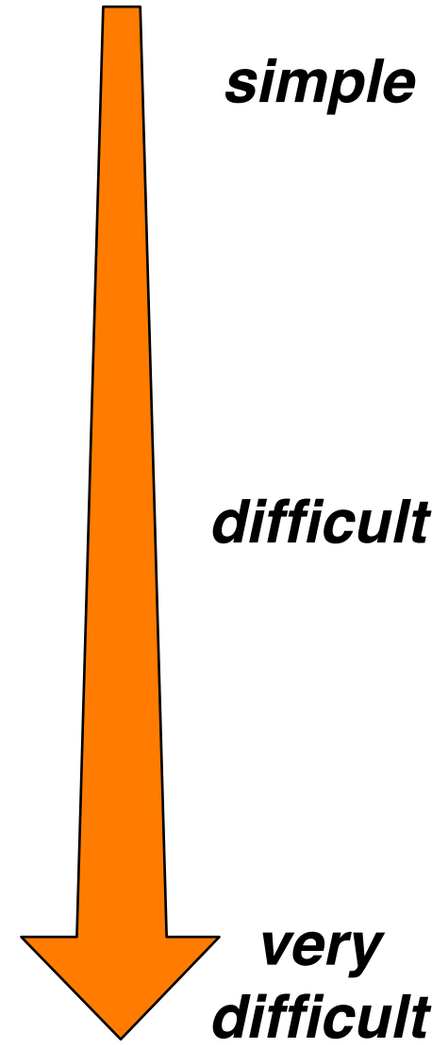
***cooperativity*** ↙ ↘ ***heterogeneity***

all binding sites are
**equivalent and not independent**

all binding sites are
**independent but not equivalent**

***heterogeneity*** ↘ ↙ ***cooperativity***

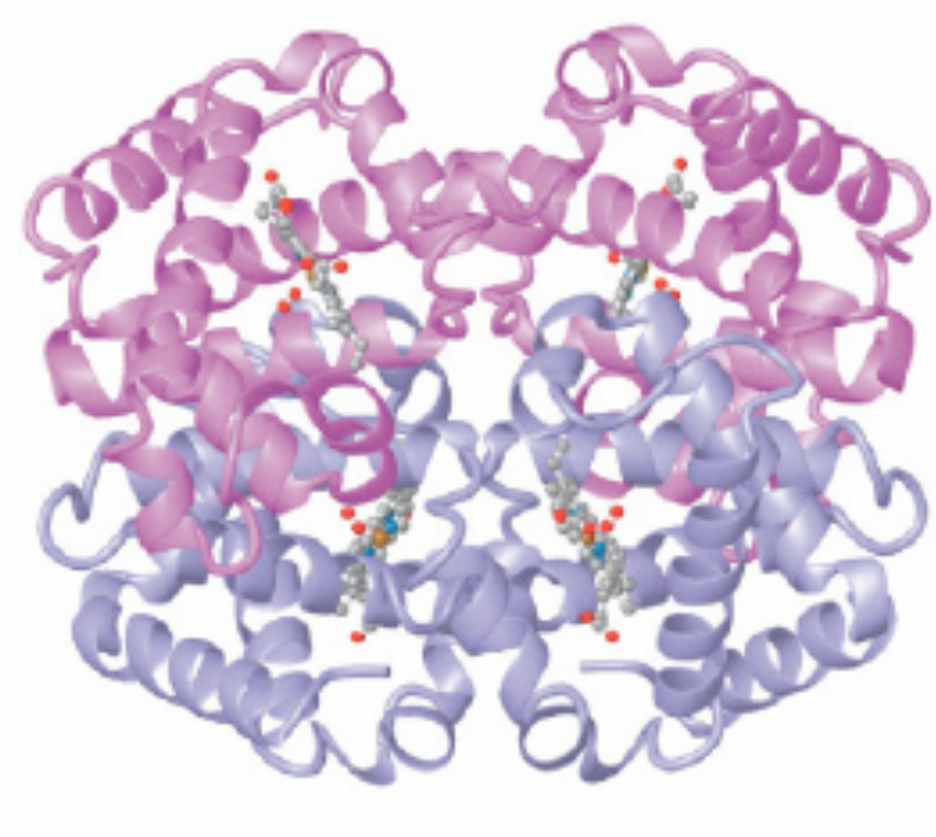
all binding sites are
**not equivalent and not independent**

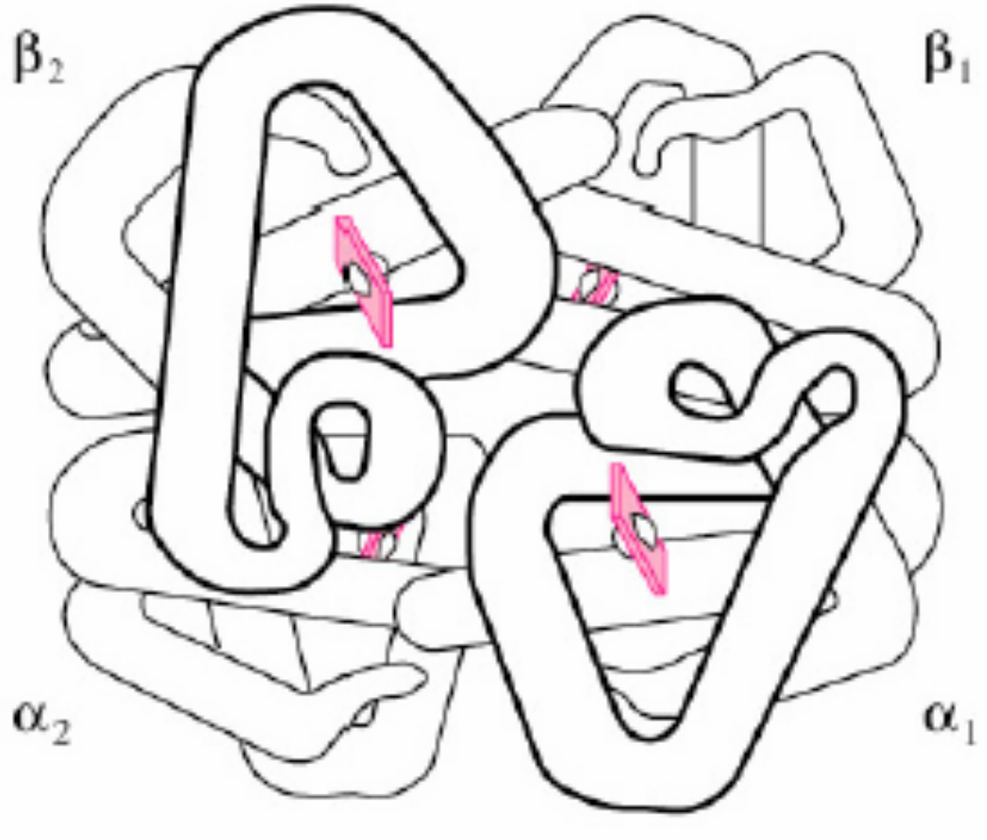


# An example for complex binding: oxygen binding to hemoglobin

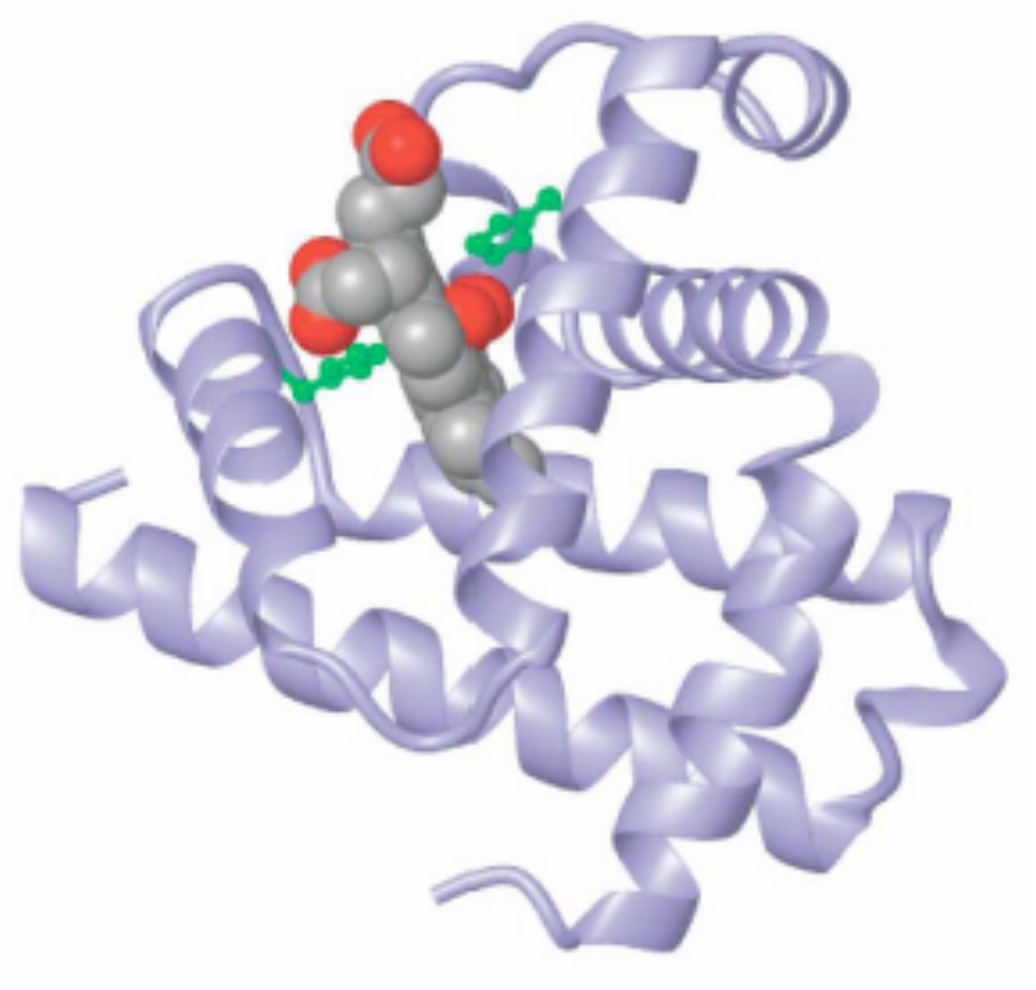
Hemoglobin

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





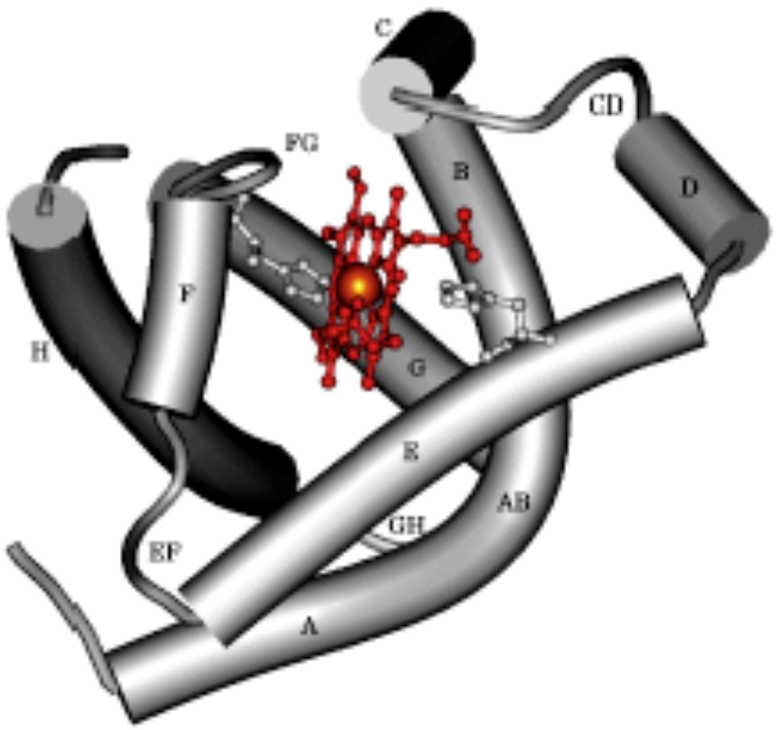
# Heme Proteins: Myoglobin and Hemoglobin

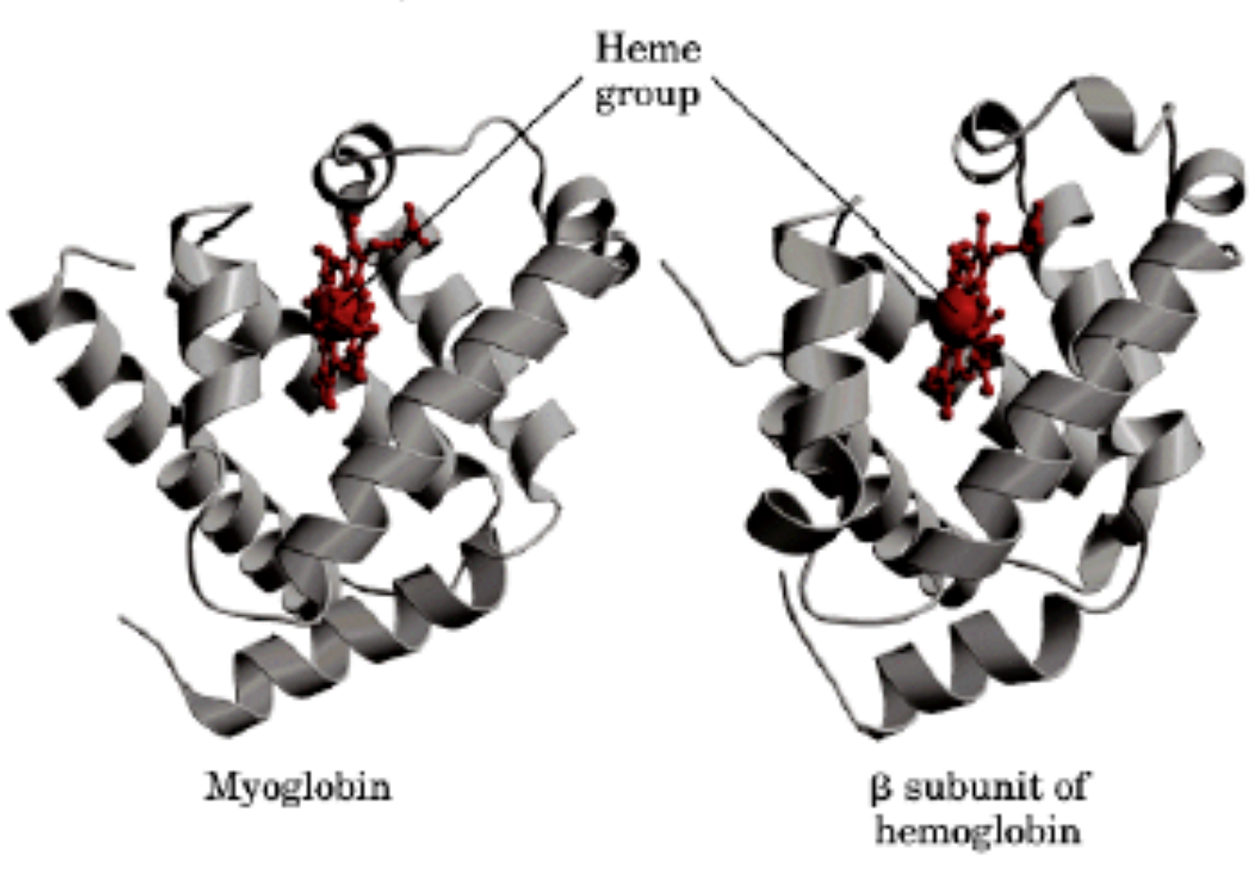


Myoglobin

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

# 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.

# Biological Uses of Cooperativity and Allostery

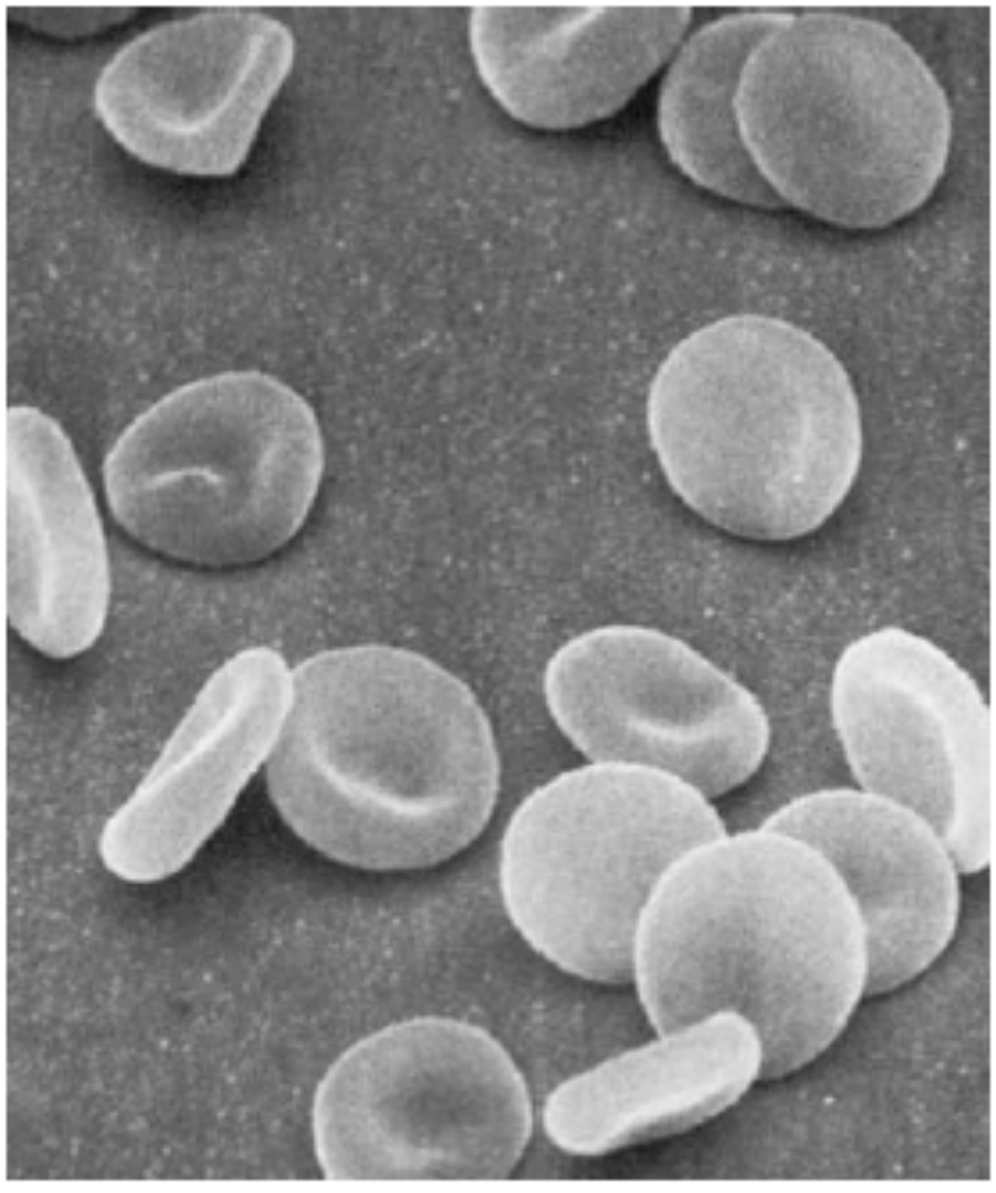
Hemoglobin: Efficient Ligand Delivery

- Hemoglobin binds $O_2$ reversibly under different partial pressures

- Why make hemoglobin cooperative?

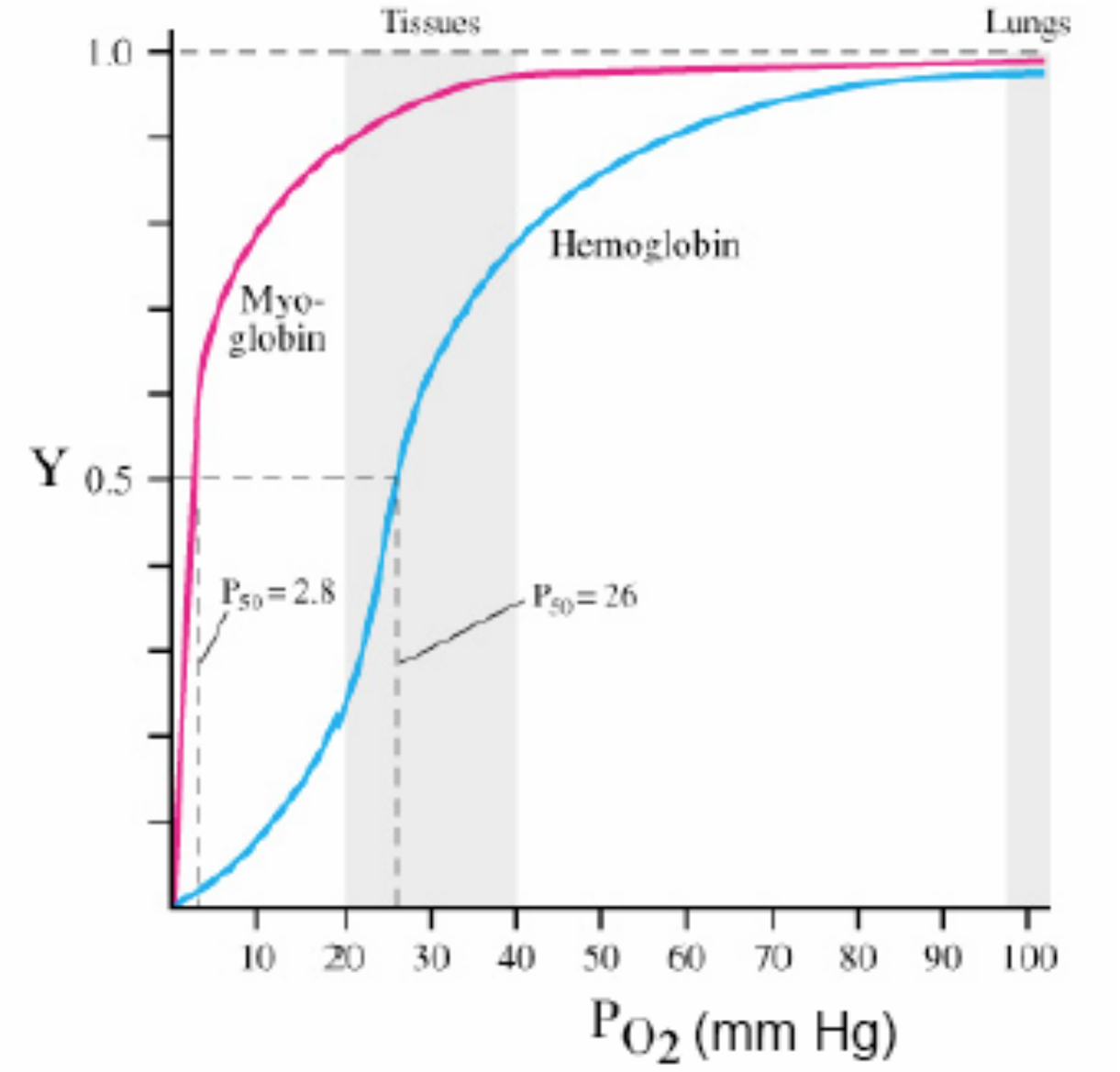
- Positive cooperativity gives all or none behavior. Thus, hemoglobin saturates at about the same $O_2$ concentration as myoglobin, but releases essentially all of its $O_2$ cargo at much higher partial pressure of $O_2$.

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 $\nu$

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

**$\nu$ for *n* binding sites (Adair equation)**

# Expression degree of binding $\nu$ for four sites

$$DP_1 <-> D + P; \quad K_1 = D \cdot P / DP_1; \quad DP_1 = D \cdot P / K_1$$

$$DP_2 <-> D + 2P; \quad K_2 = D \cdot P^2 / DP_2; \quad DP_2 = D \cdot P^2 / K_2$$

$$DP_3 <-> D + 3P; \quad K_3 = D \cdot P^3 / DP_3; \quad DP_3 = D \cdot P^3 / K_3$$

$$DP_4 <-> D + 4P; \quad K_4 = D \cdot P^4 / DP_4; \quad DP_4 = D \cdot P^4 / K_4$$

# Expression degree of binding ν for four sites

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

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

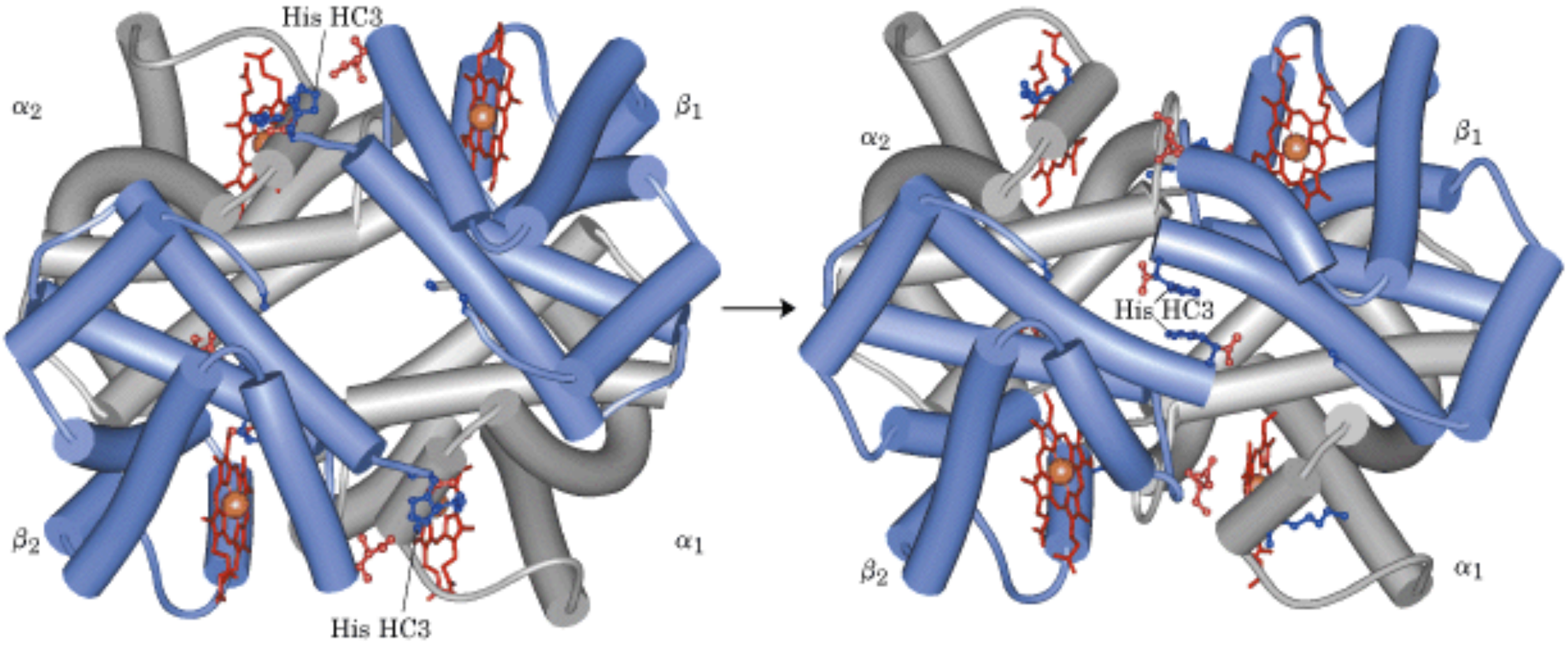
$$\nu_4 = \frac{\frac{1}{K_1} \cdot P_{\text{free}}^1 + \frac{2}{K_2} \cdot P_{\text{free}}^2 + \frac{3}{K_3} \cdot P_{\text{free}}^3 + \frac{4}{K_4} \cdot P_{\text{free}}^4}{1 + \frac{1}{K_1} \cdot P_{\text{free}}^1 + \frac{1}{K_2} \cdot P_{\text{free}}^2 + \frac{1}{K_3} \cdot P_{\text{free}}^3 + \frac{1}{K_4}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

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

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

**T** Deoxy (tense) state
with low binding affinity

**R** Oxy (relaxed) state
with high binding affinity

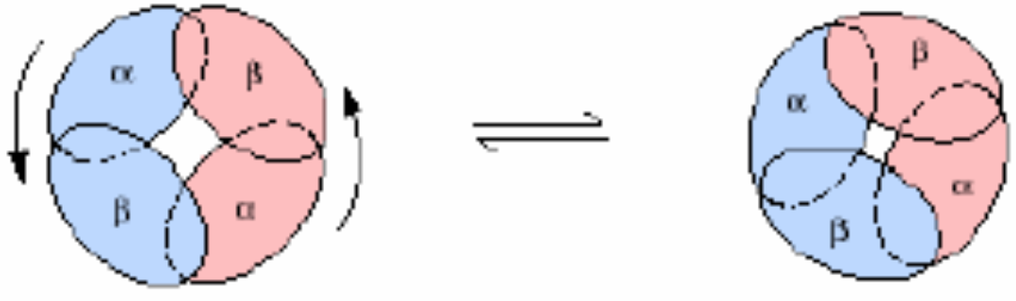



**T state** **R state**

# $O_2$ binding to the heme effects the entire hemoglobin structure.

**T** Deoxy (tense) state
with low binding affinity

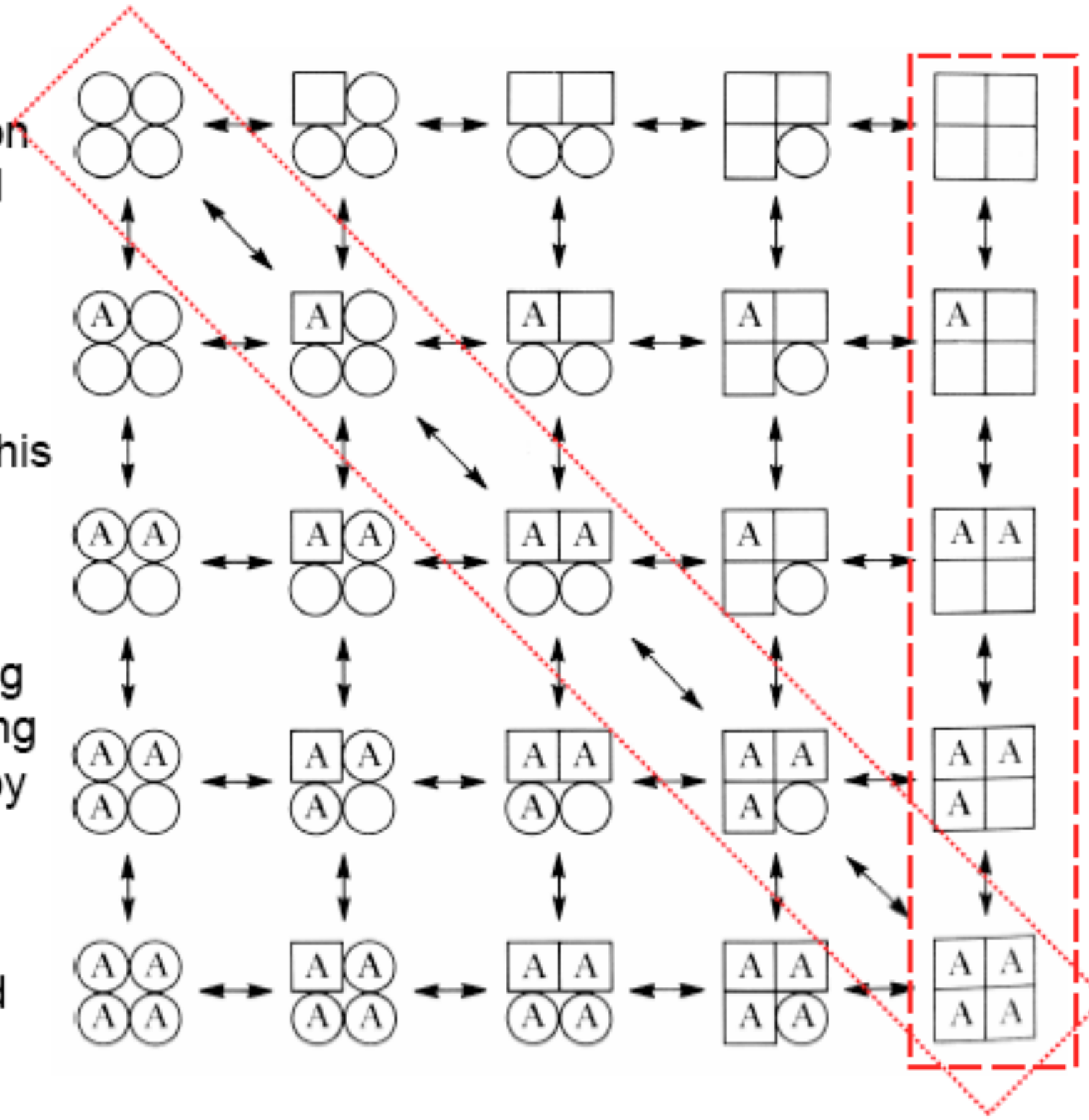
**R** Oxy (relaxed) state
with high binding affinity



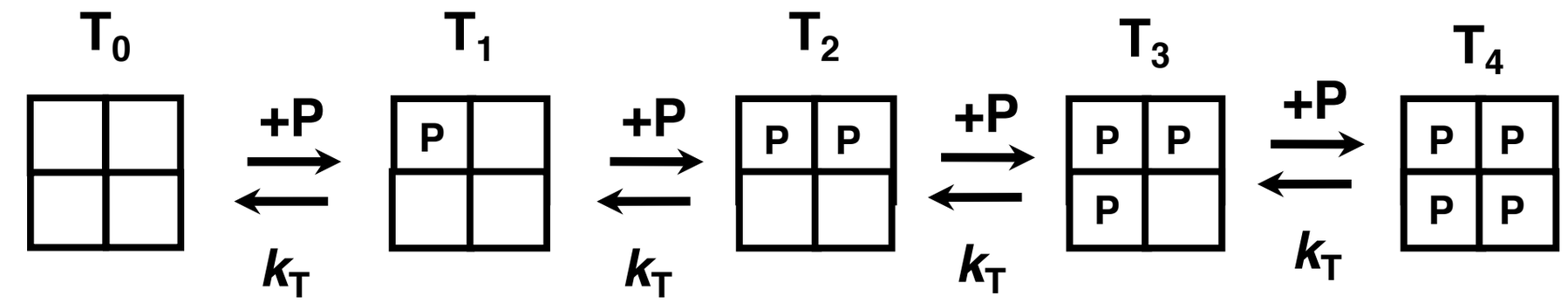

- $O_2$ binding causes a series of shifts in all subunits, one αβ pair rotates and slides with respect to the other pair.
- There is a change in the heme structure upon binding $O_2$.
- Since His F8 is covalently attached to the heme, all of helix F shifts.
- The reorganization of helix F alters the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

# A more general allosteric scheme...

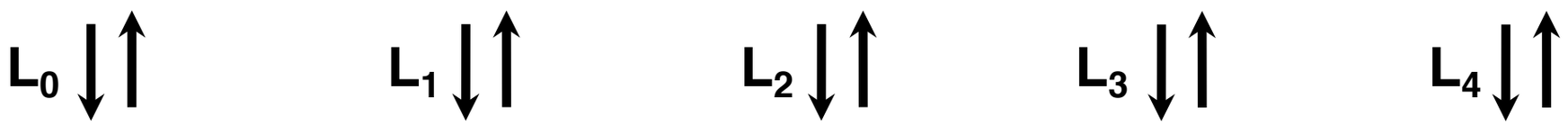
- This scheme allows the individual subunits to take on either of two conformational forms, regardless of the number of ligands that are bound.
- For a four-subunit protein, this allow 25 different combinations.
- The MWC model is a limiting case of this scheme involving only the species enclosed by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

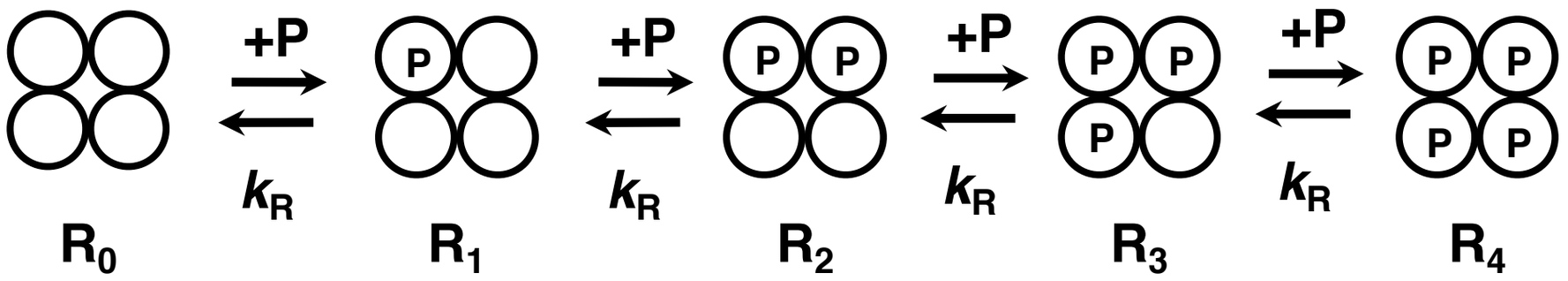


# The Monod-Wyman-Changeau (MWC) model for cooperative binding




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

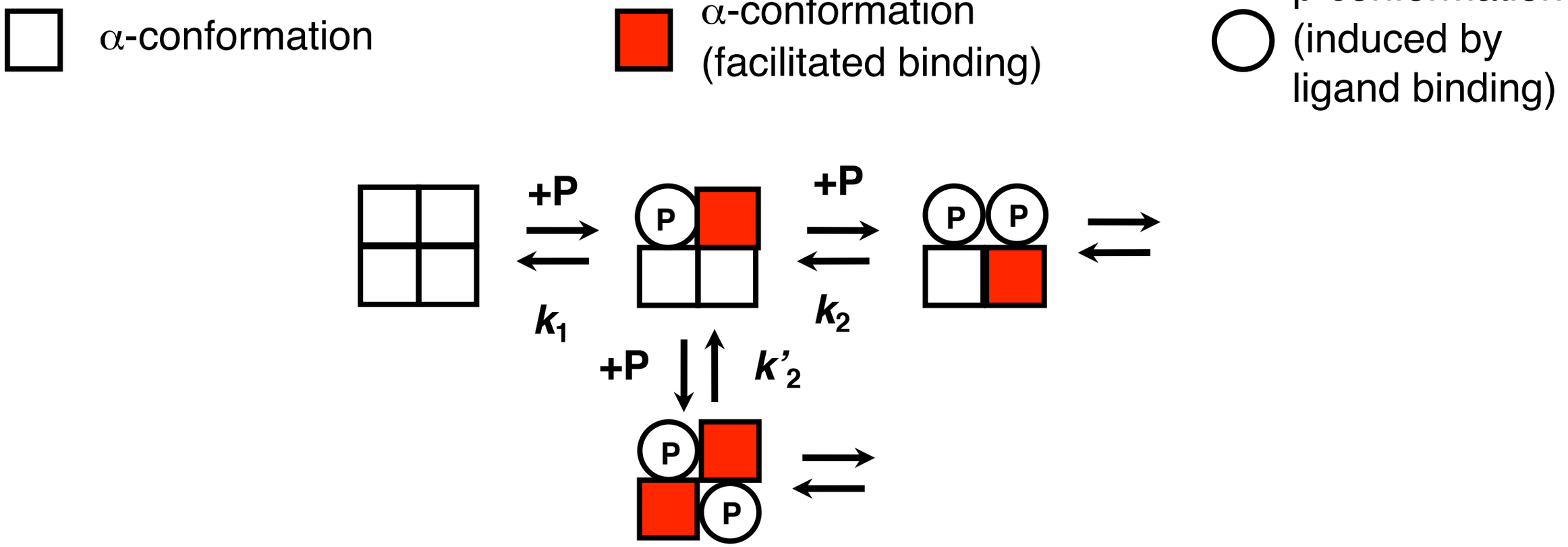
$$c = K_R/K_T$$

ratio of binding constants for R and T states

$$\alpha = [X]/K_R$$

normalized ligand concentration

# 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.

# 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 $K_1$

$D_{\text{free}}$, concentration free DNA; $P_{\text{free}}$, 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 $K_2$

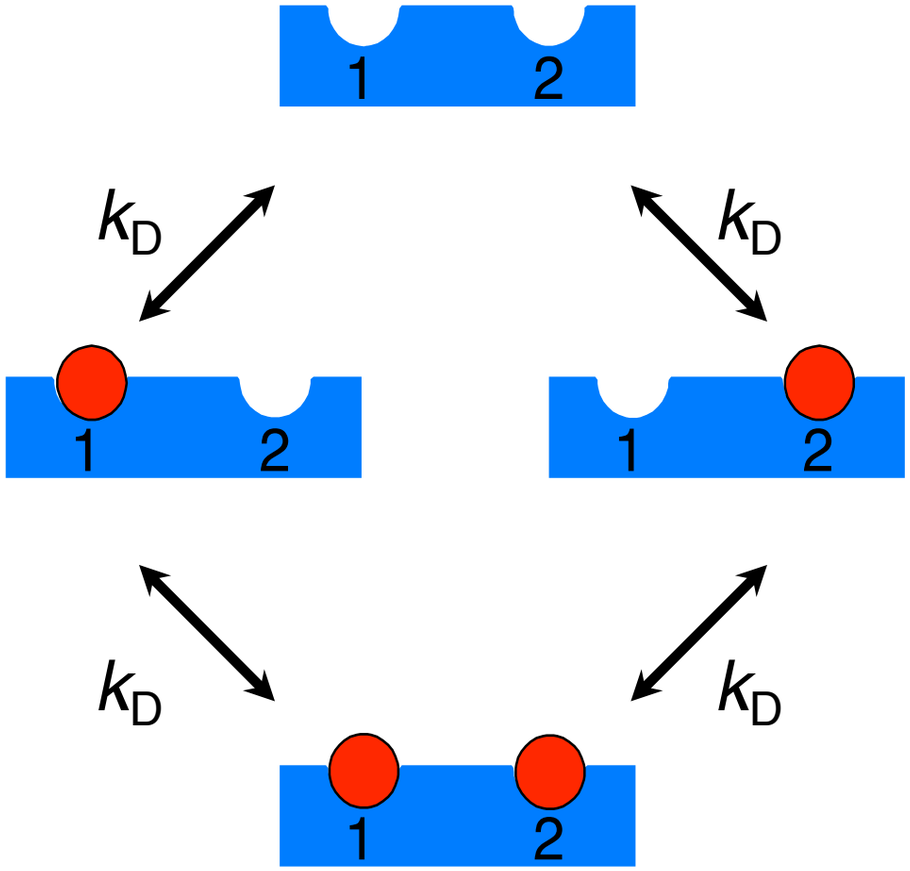
$$D + 2P_{\text{free}} \rightleftharpoons DP_2 \qquad K_2^* = \frac{D_{\text{free}} \cdot P_{\text{free}}^2}{DP_2} \qquad K_2^* = K_1 \cdot K_2$$

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*

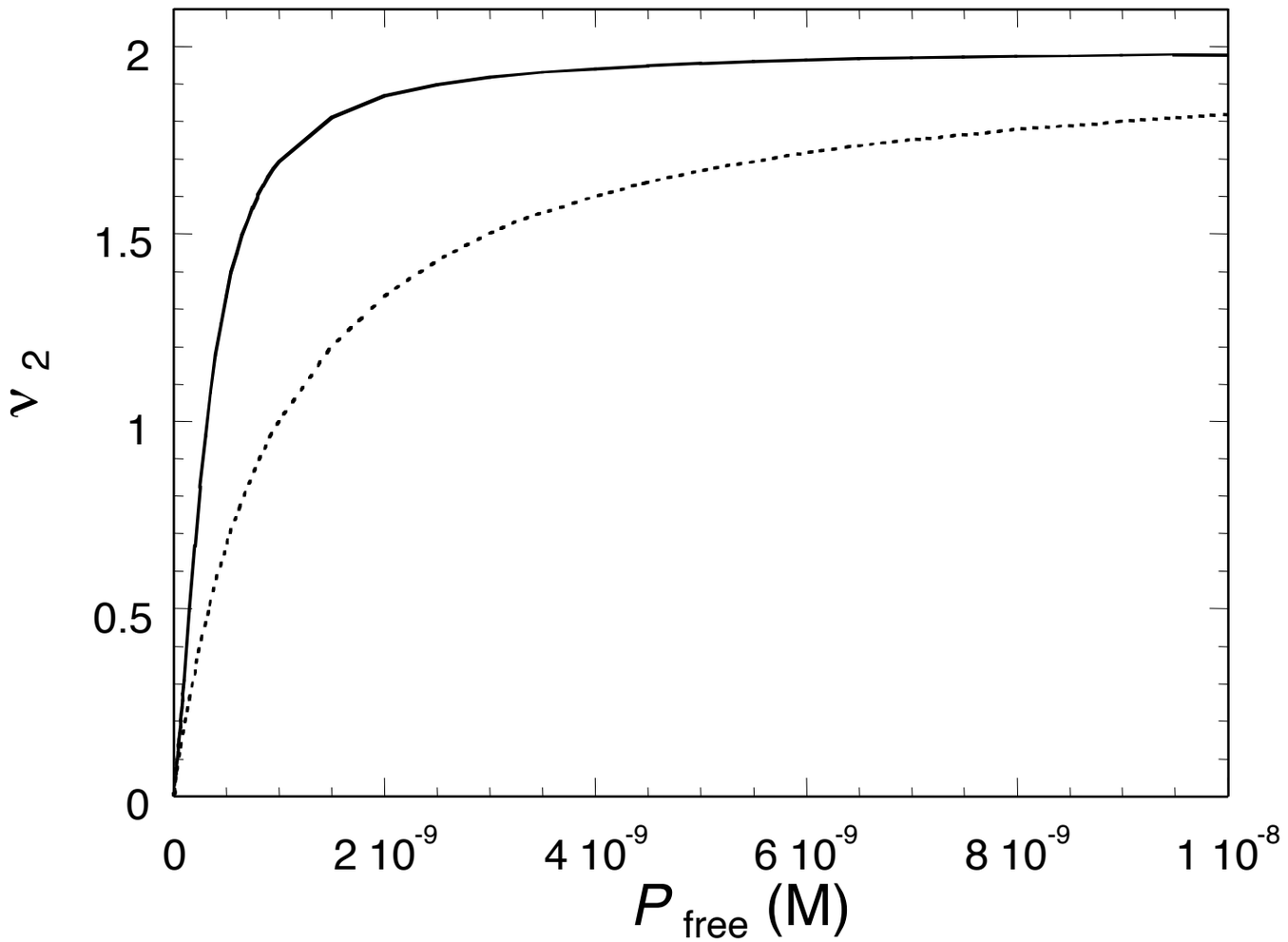
2 possibilities for the dissociation of $DP_2$

$$K_2 = 2k_d$$

$DP_2$

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

# 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)

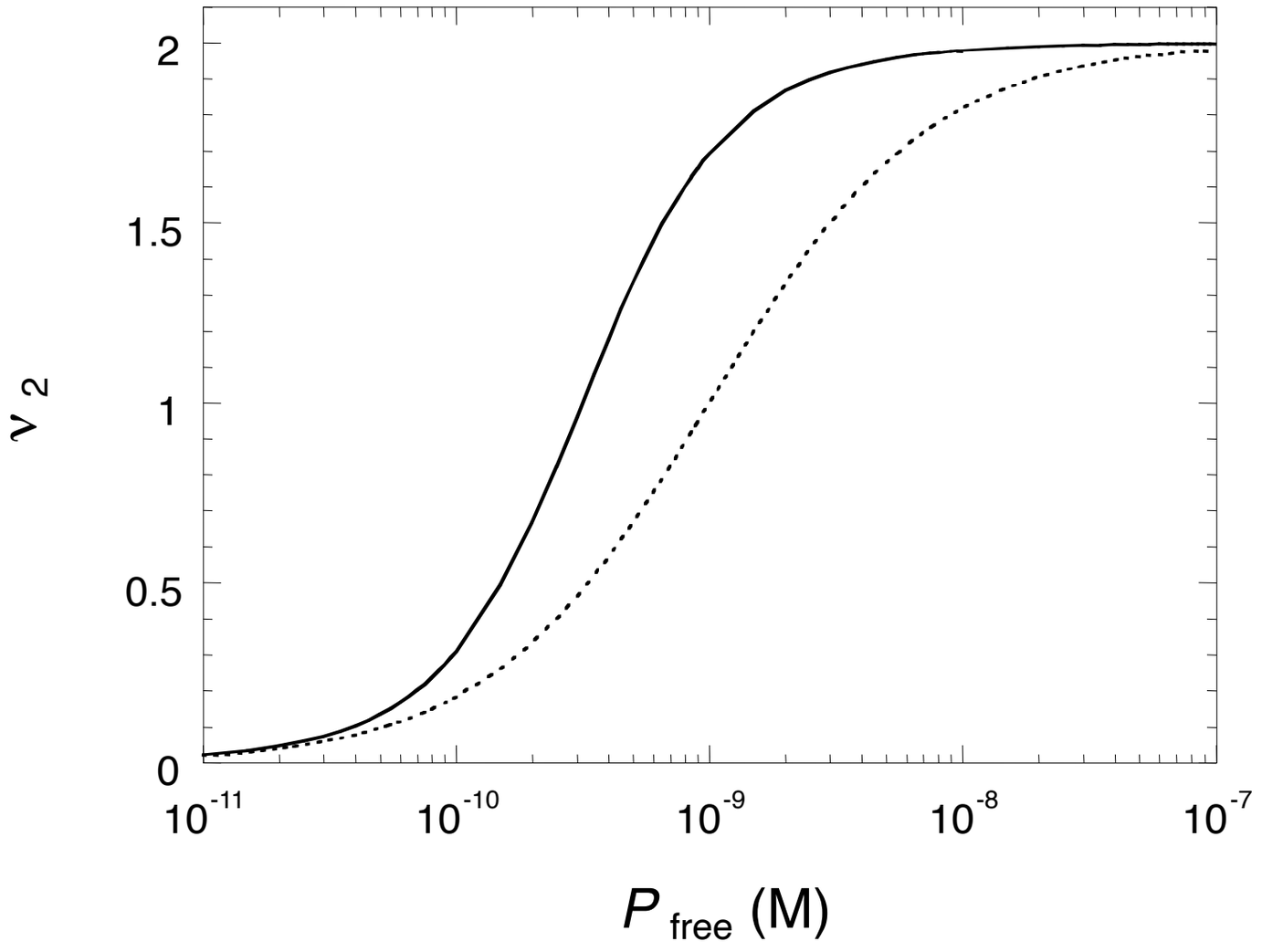
**cooperative binding**
microscopic binding constant
$k_D = 10^{-9}$ (M)

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 $\mu$ 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

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

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, $\alpha_{\text{H}}$ 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, $\alpha_H$ HiIl coefficient

$L_{\text{free}}$ is free ligand

The HiIl $\alpha_H$ 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

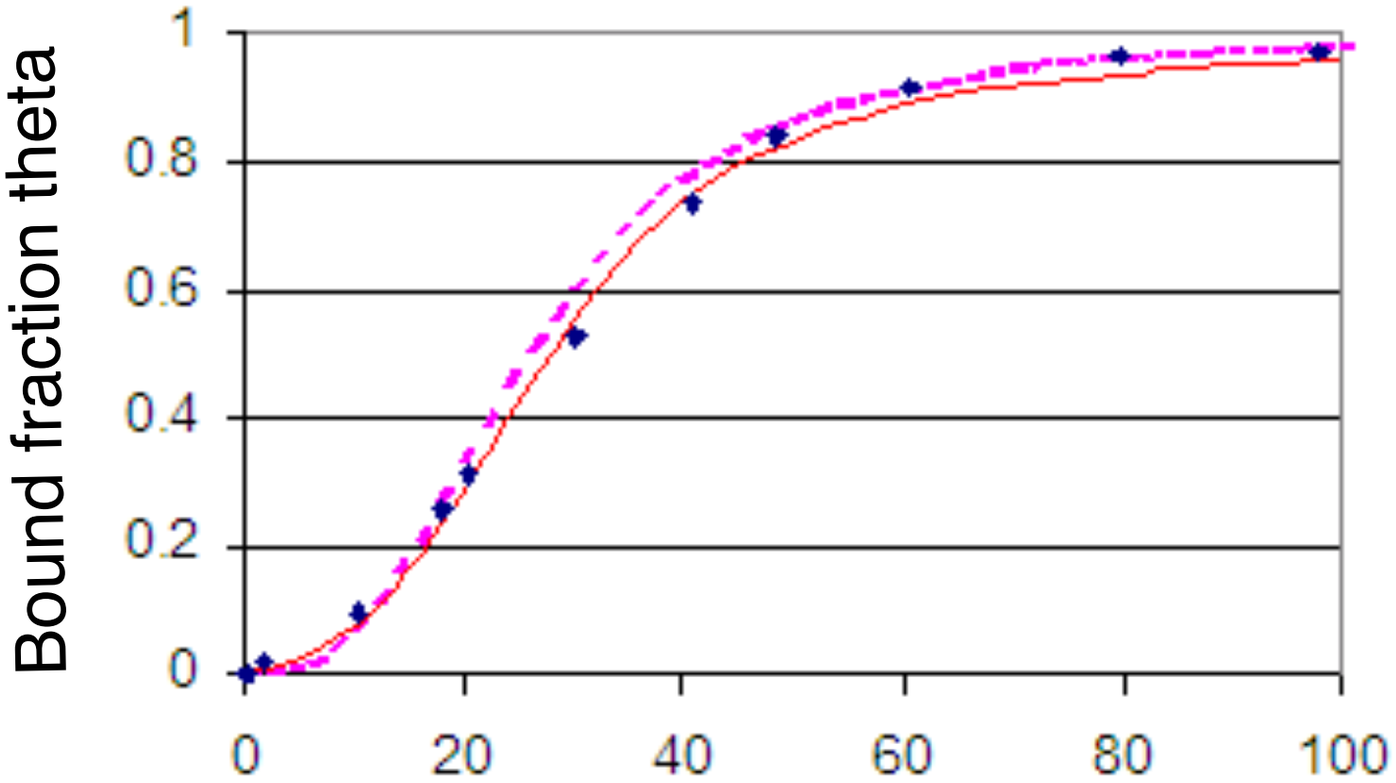
$\alpha_H = n$, the cooperativity is infinite

$\alpha_H = 1$, 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

# Fit of experimental data for binding of $O_2$ to hemoglobin




***L*** (free/normalized ligand concentration; $pO_2$ in torr)

$$\theta = \frac{L_{\text{free}}^{\alpha_H}}{K^{\alpha_H} + L_{\text{free}}^{\alpha_H}} \qquad \alpha_H = 2.8$$

$$\theta = \frac{R\,c\,L(1+c\,L)^{n-1} + L(1+L)^{n-1}}{(1+L)^n + R(1+c\,L)^n\,n}$$

MWC model ($n$ = 4)
R = 9000
c = $K_R/K_T$ = 0.014
$K_R$ = 2.8

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

# Hill coefficient and Hill plot

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

$\alpha_H$ HIll coefficient

$L_{\text{free}}$ is free ligand

$K$ average microscopic binding constan

rearrange the terms to get

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

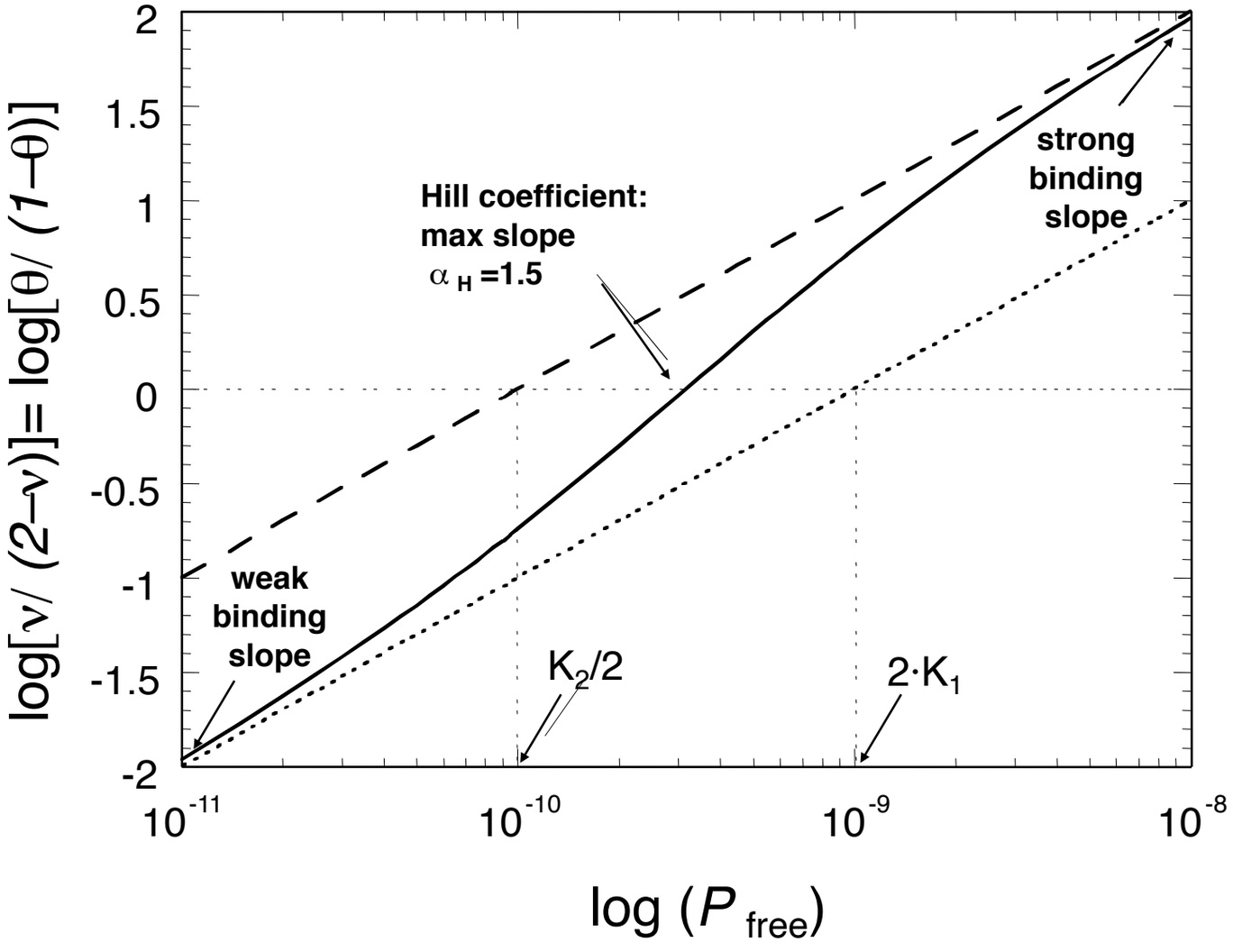
which yields the Hill equation

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

# Visualization of binding data - Hill plot

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

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

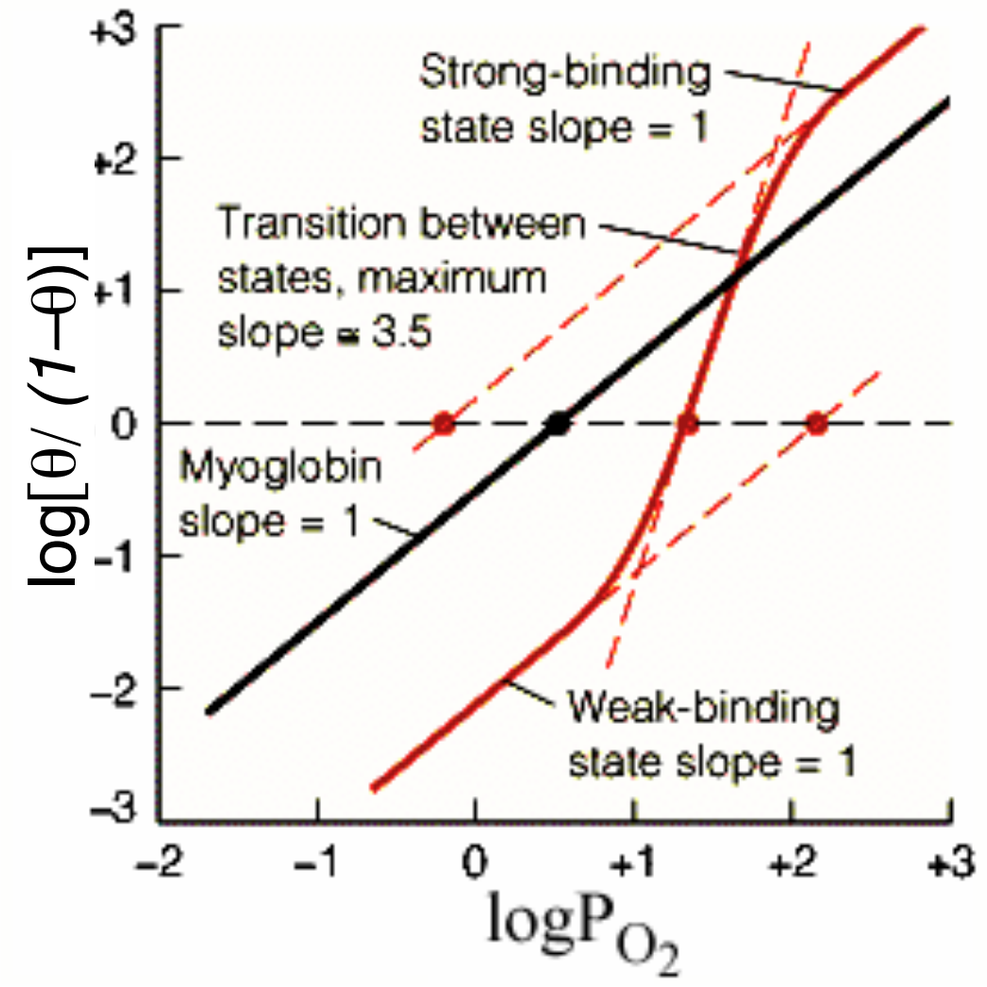



$$P_{free} \to \infty \Rightarrow \log\left(\frac{\nu_2}{2-\nu_2}\right) = \log\left(P_{free}\right) - \log\left(\frac{K_2}{2}\right)$$

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

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

# Hill Plots for Oxygen Binding to Hemoglobin and Myoglobin

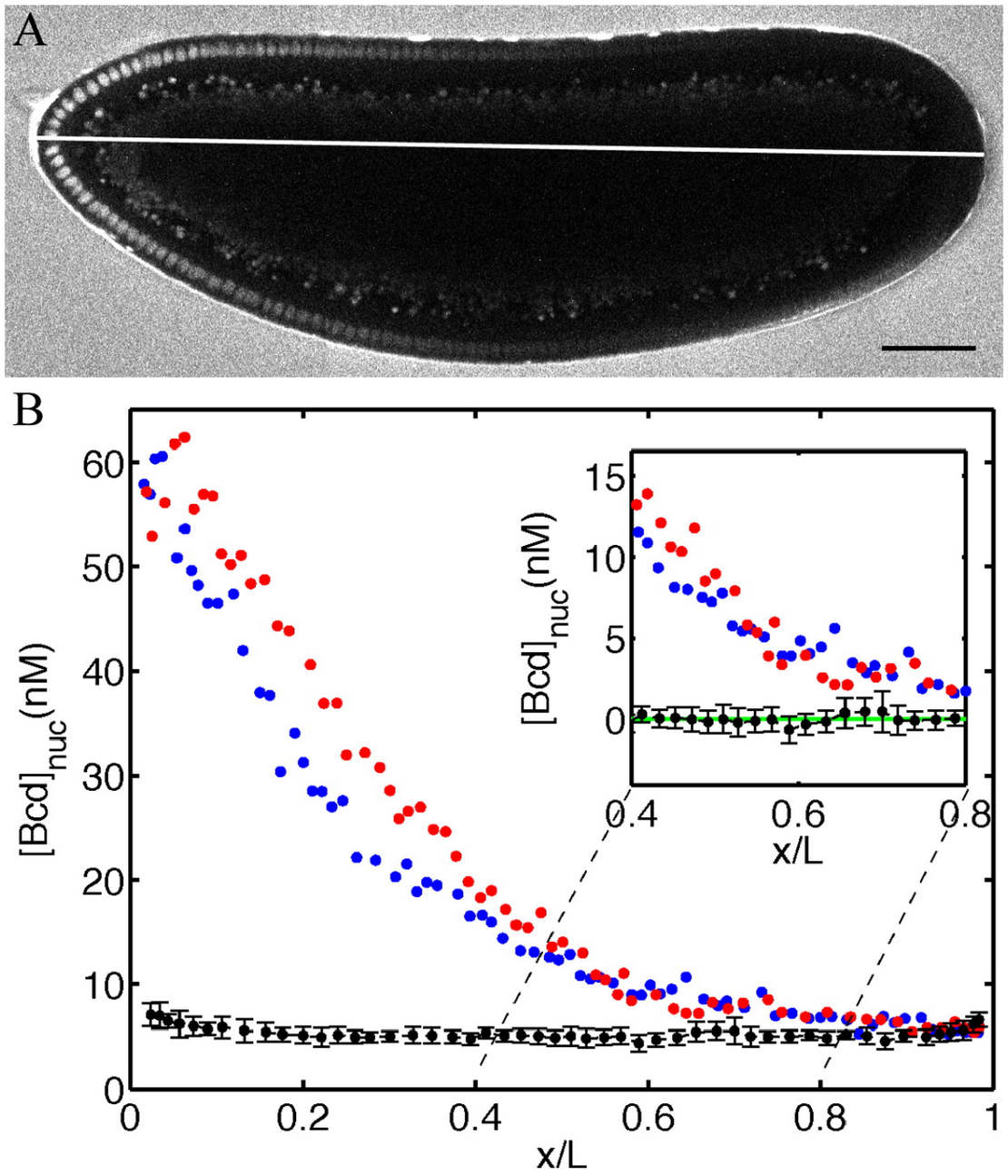



- At low $P_{O_2}$ , the Hill plot has a slope = 1 and corresponds to the weak binding state (large $P_{50}$)
- As binding progresses, the curve switches over to approach another parallel straight line that describes the strong binding state (small $P_{50}$).
- The transition between binding states is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

## Why isn't the Hill plot linear?

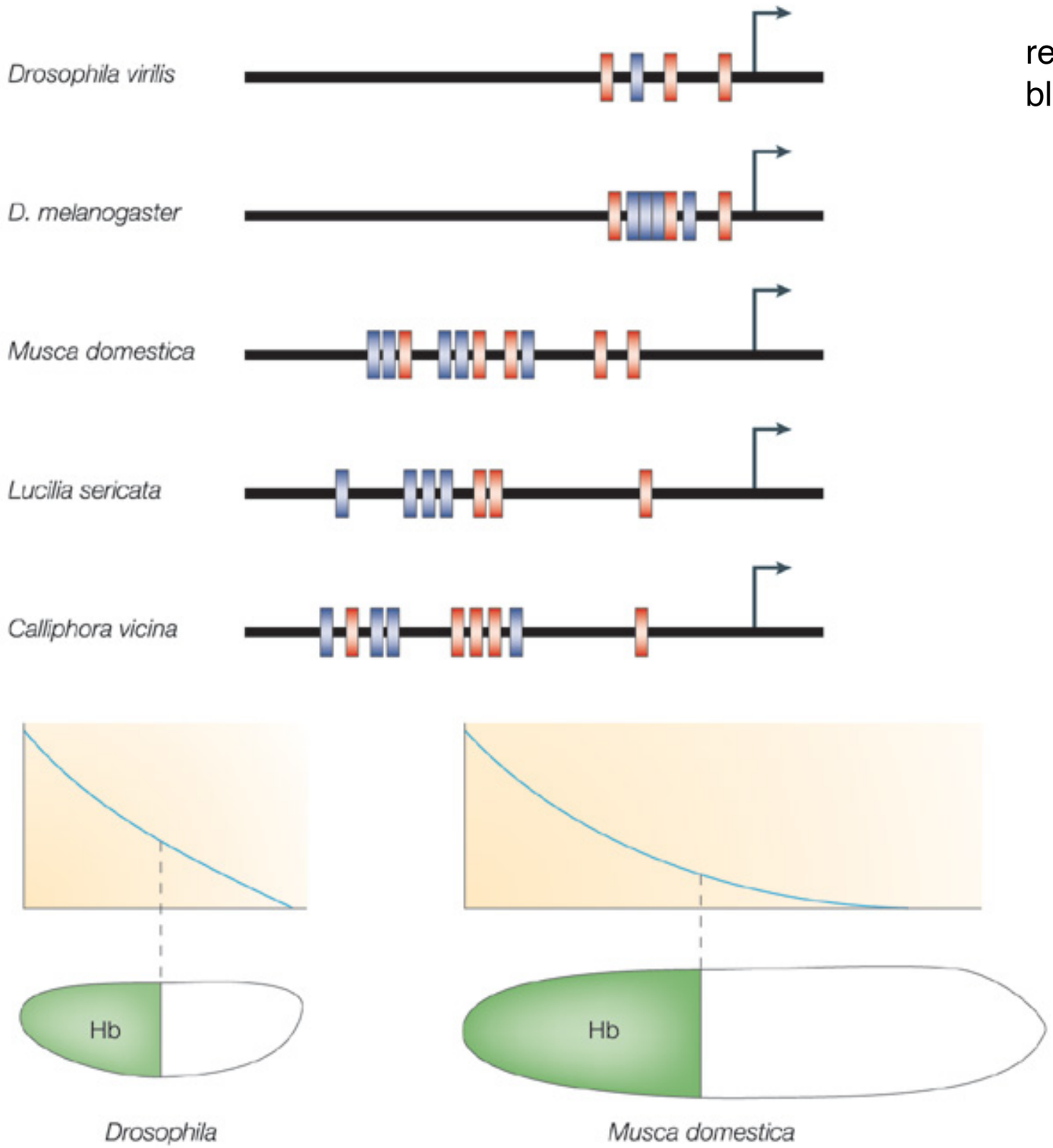
- When cooperativity is not complete (i.e., $n_h < N$), the Hill plot is not linear.
- At the extremes of [L], the line has a slope of ~1.0.
- At low ligand concentrations, there is no cooperativity. Thus the Hill plot will represent single-site binding (binding of the first ligand molecule).
- At high 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.

# 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.

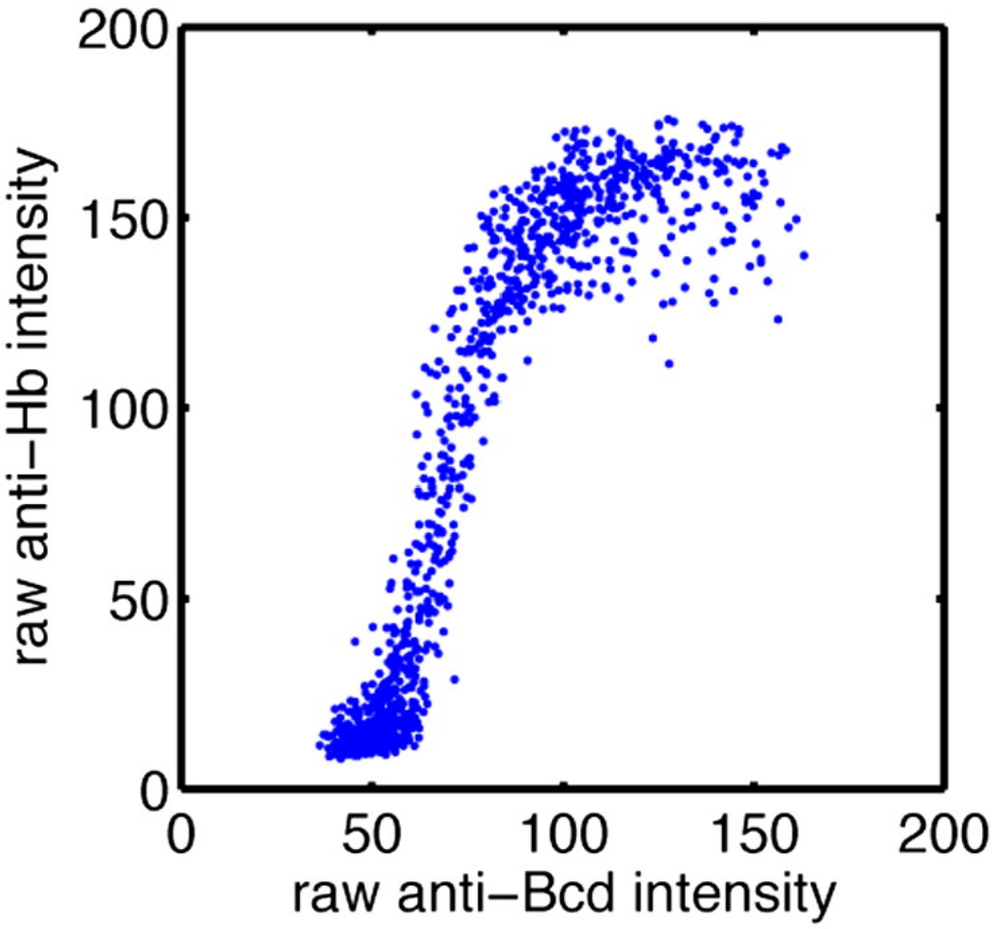
# Number and distribution of Bicoid-binding sites in the P2 promoter of hunchback (hb) of five species of flies.

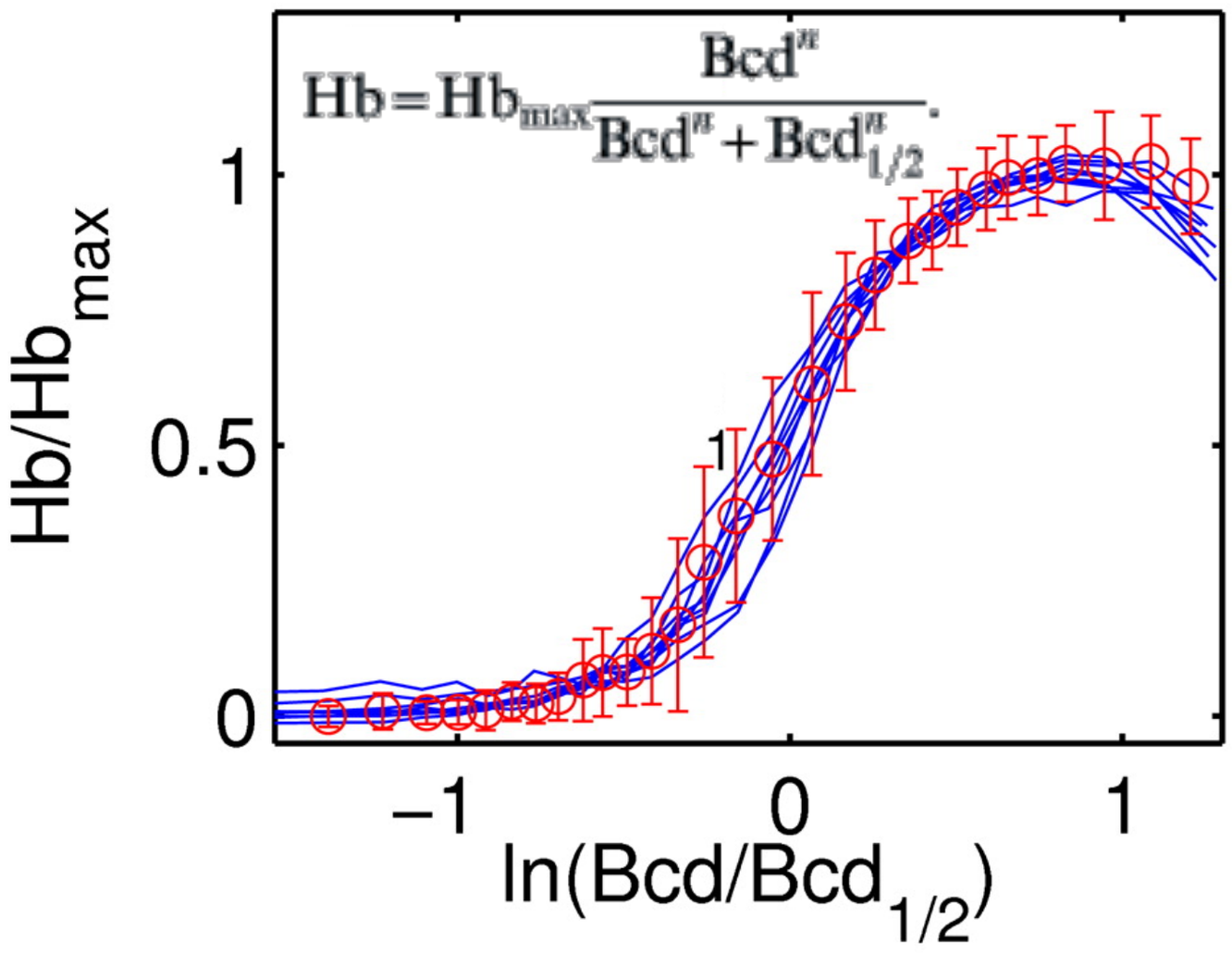



red: strong binding sites
blue: weak binding sites

Simpson 2002, Nat Rev Genet 3, 907

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

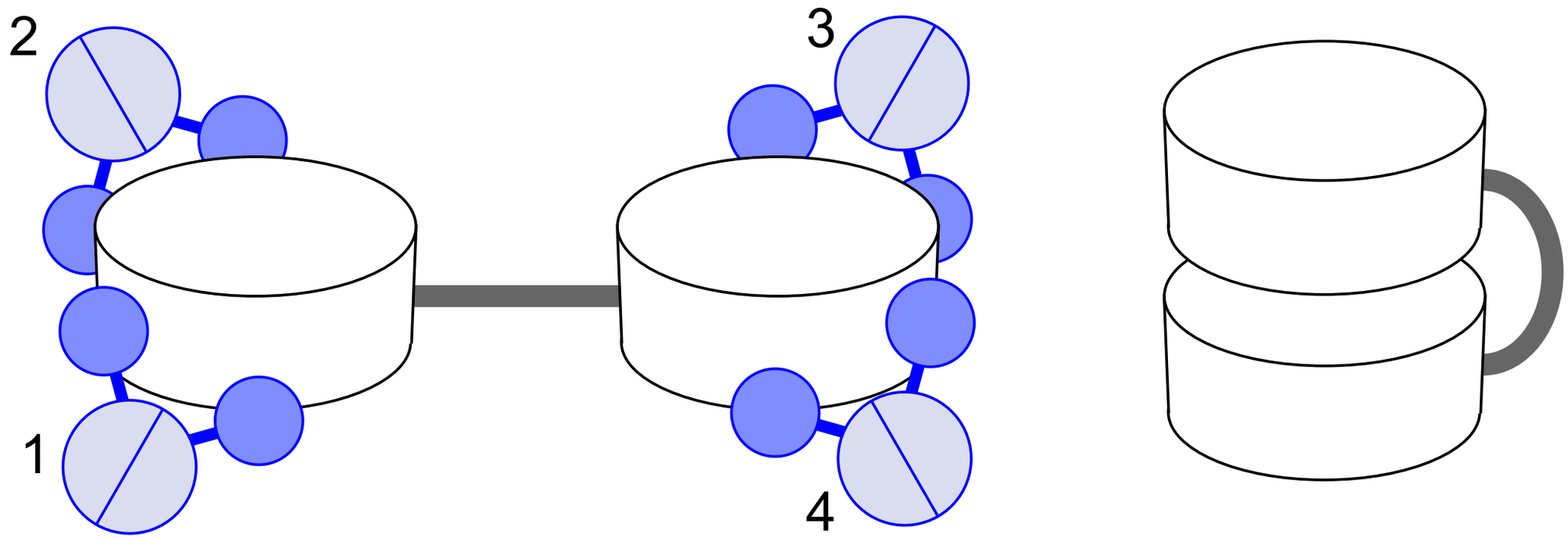


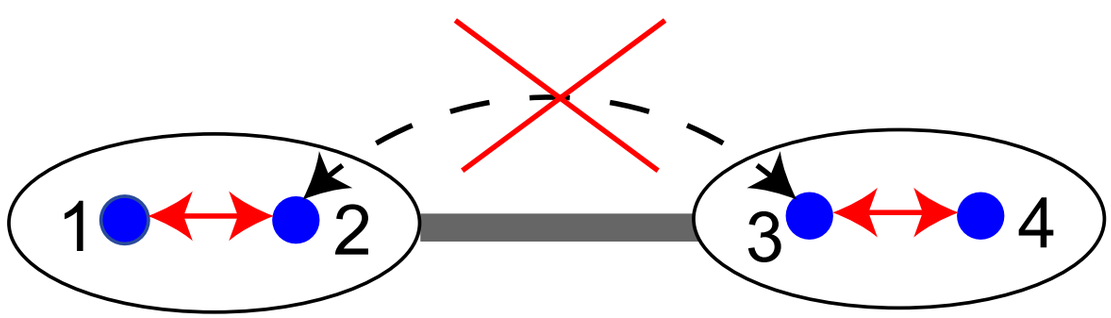



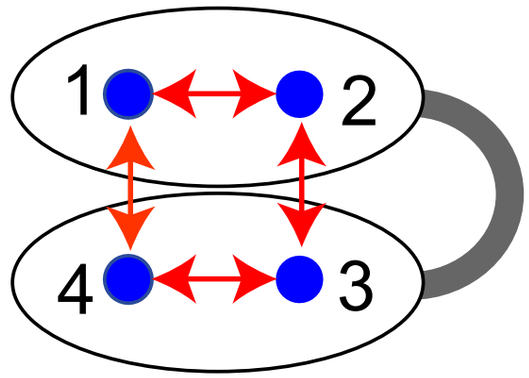

Hb transcription is activated by cooperative binding of Bcd molecules for which 7 Bcd-binding sites are present at the *hb* promoter

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

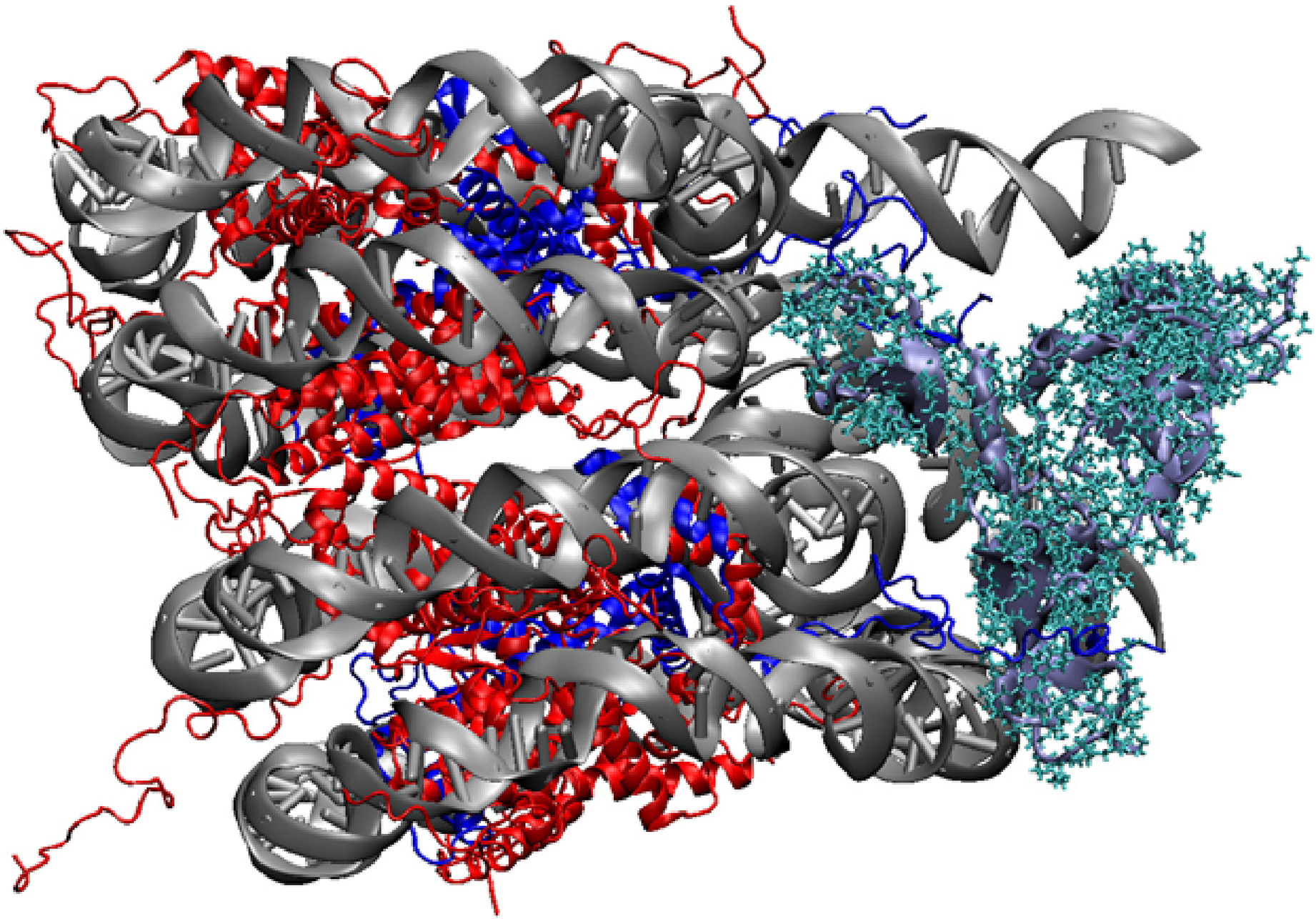
# Binding of heterochromatin protein 1 (HP1/Swi6) to chromatin



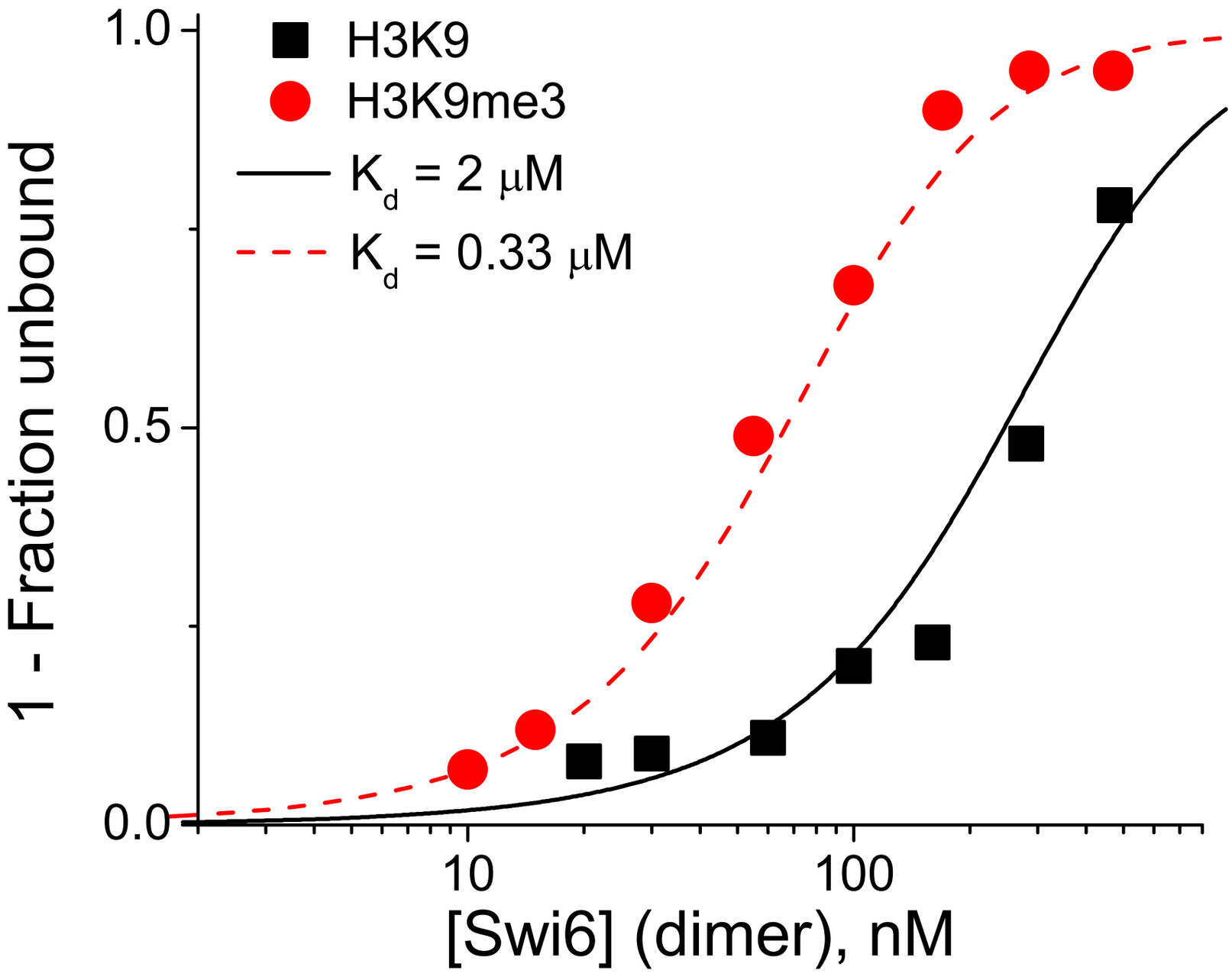



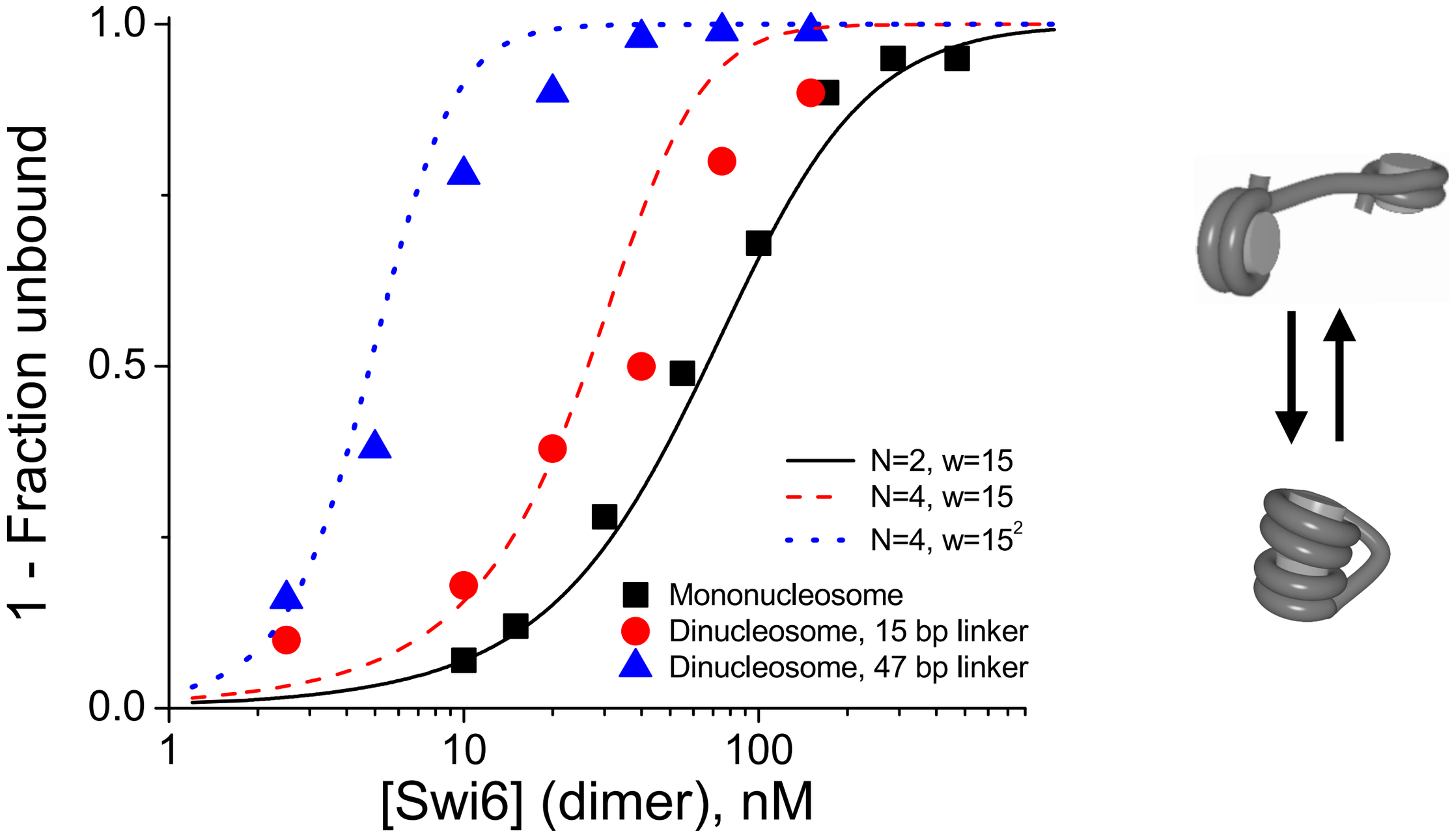
# Binding model of Swi6 dimer with two stacked nucleosomes



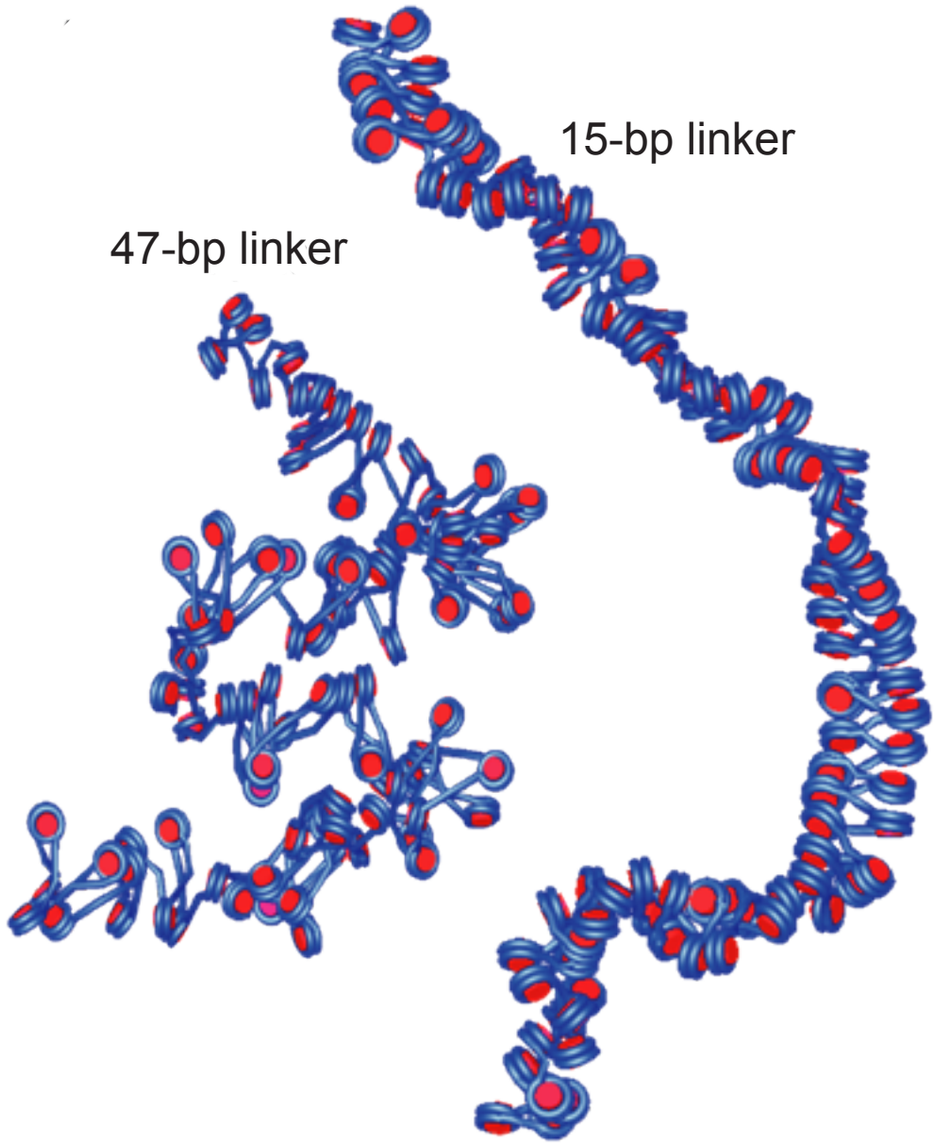
# Binding affinity of Swi6 to a mononucleosome depends on methylation a lysine 9 of histone H3 (H3K9me3)



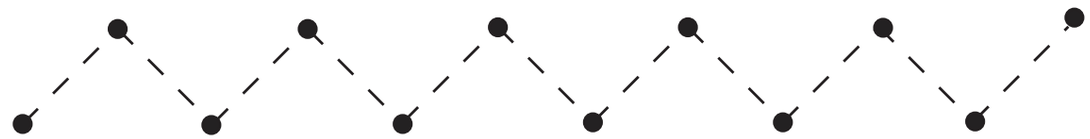
# Nucleosome conformation determines cooperativity/allostery



# Spatial folding of the nucleosome chain is linked to protein binding

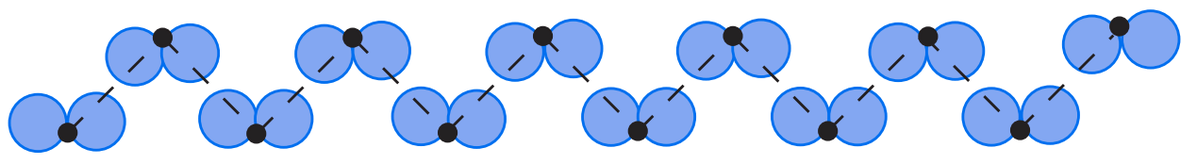



12-nucleosome zigzag lattice:

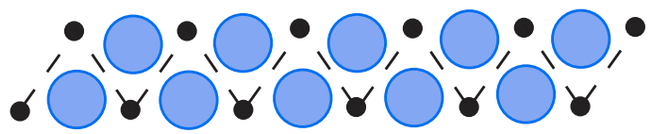


- HP1 dimer

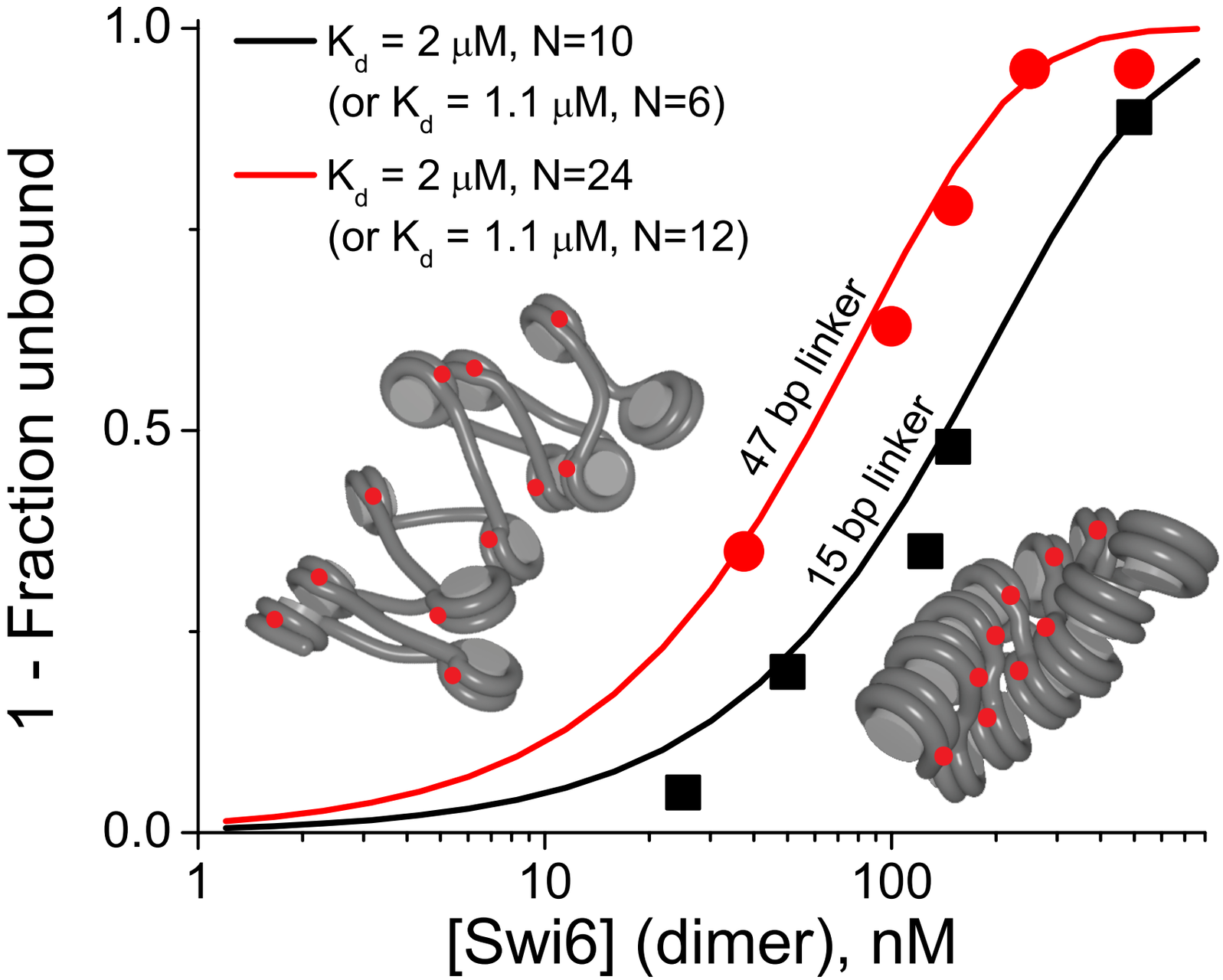
12-nucleosome lattice with long linkers: 24 HP1 dimers



12-nucleosome lattice with short linkers: 10 HP1 dimers



# Binding curve difference might reflect stoichiometry differences



# Summary

Thermodynamic equilibrium: $\Delta G$, $K_D$ 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; occupancy: $K_B$ x *concentration*

Ways to visualize binding curves:

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

Parameters and models:

- stoichiometry/number of binding sites
- microscopic/macroscopic affinity, cooperativity, heterogenity, allostery
- independent binding, all-or-none binding, Hill coefficient for complex binding
- MWC and KNF model for allosteric binding

# The “simple” Michaelis-Menten reaction

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} \mathrm{ES} \underset{k_{-2}}{\overset{k_{+2}}{\rightleftarrows}} \mathrm{E} + \mathrm{P}$$

$e_0 - x \qquad s_0 - x - p \qquad x \qquad p$

$$\frac{dx}{dt} = k_{+1} \cdot (e_0 - x) \cdot (s_0 - x - p) - k_{-1} \cdot x - k_{+2} \cdot x + k_{-2} \cdot (e_0 - x) \cdot p$$

$$\frac{dp}{dt} = k_{+2} \cdot x - k_{-2} \cdot (e_0 - x) \cdot p$$

The second equation can be used to express x and dx/dt in dependence of p and dp but the resulting equation has no solution in p and t

⇒ simplifications like s0 >> e0 or dx/dt = constant

# The “simple” Michaelis-Menten reaction

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P} \qquad (9)$$

simplifications like S0 >> E0 and ES concentration = constant

$$K_\mathrm{M} = \frac{k_{-1} + k_2}{k_1} \qquad V_{\max} = k_2[\mathrm{E}]_\mathrm{T} \qquad (22)$$

$$V_0 = V_{\max} \frac{[\mathrm{S}]}{[\mathrm{S}] + K_M} \qquad (23)$$

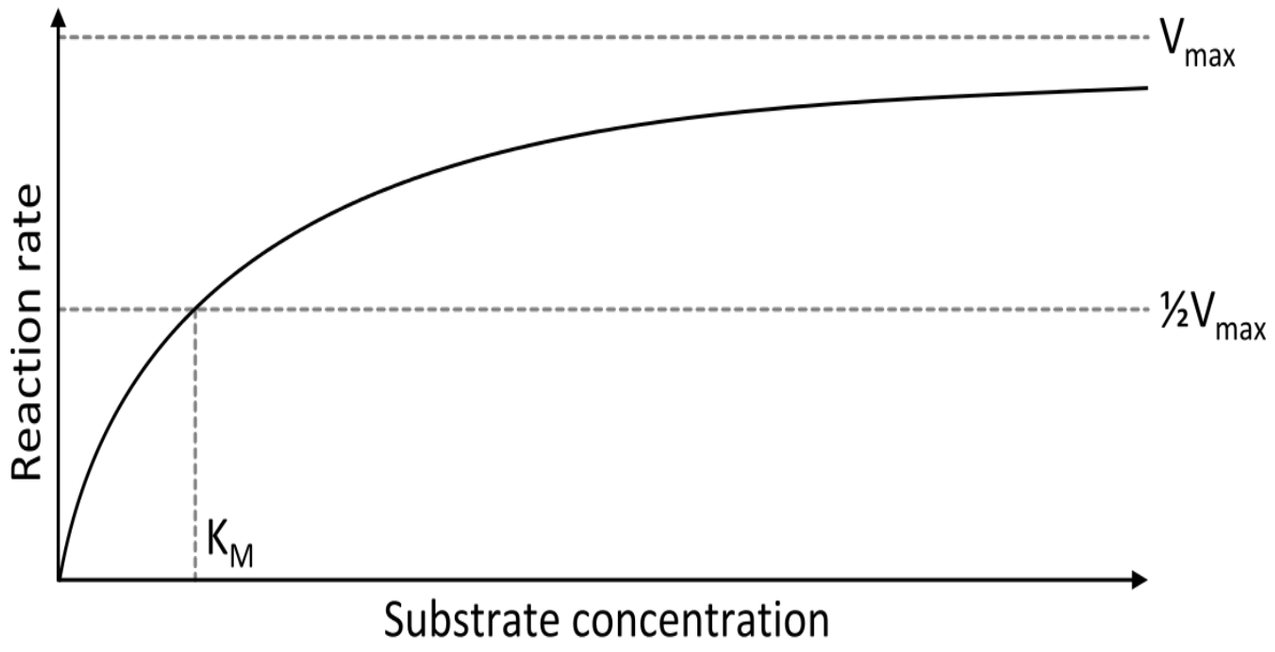
$$\nu_1 = \frac{P_{\mathrm{free}}}{P_{\mathrm{free}} + K_\mathrm{D}}$$

binding to a single binding site

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

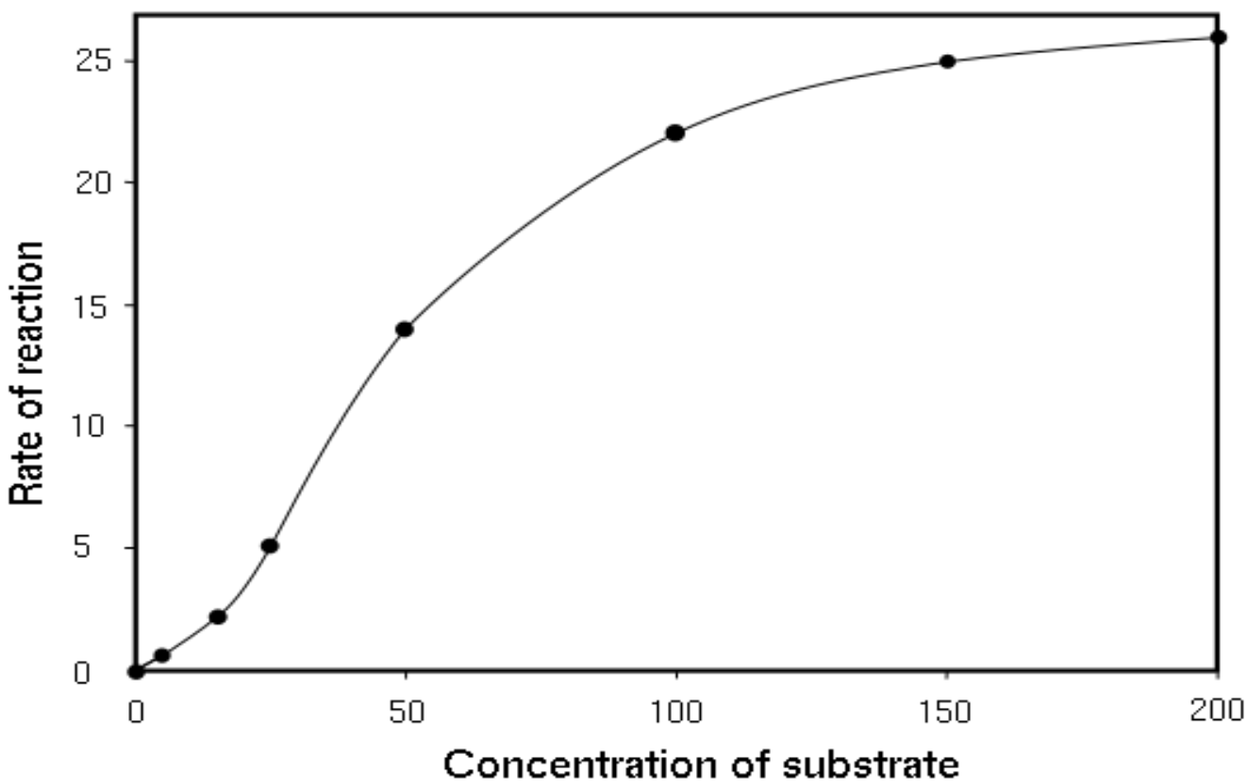
Hill equation

# Michaelis Menten kinetics




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

$$K_{\text{M}} = \frac{k_{-1} + k_2}{k_1}$$ for k2 << k-1




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