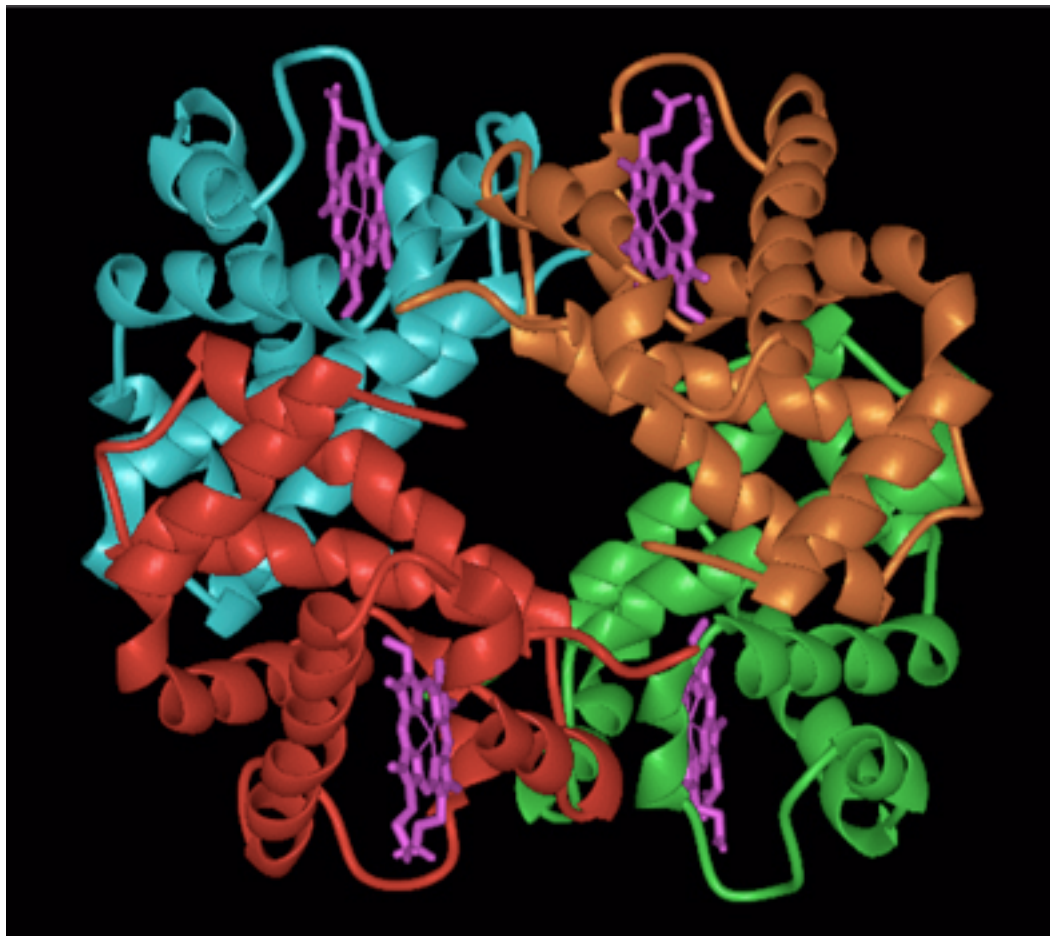


Statistical Thermodynamics: Biological Regulation through Allostery

Based on Cantor & Schimmel,
Chapt. 17

COOPERATIVE OXYGEN BINDING PROPERTIES OF HEMOGLOBIN

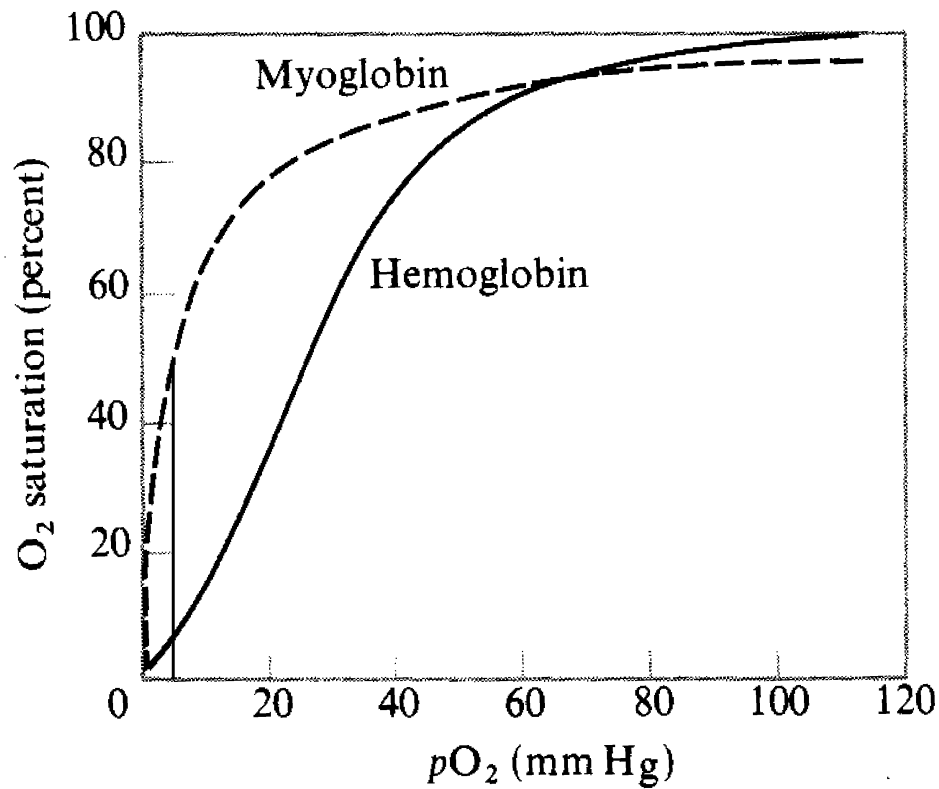
Hemoglobin is a heterotetramer consisting of two α and two β subunits:



Each subunit ~150 residues

Each subunit has a heme with
an Fe^{2+} atom

Hemoglobin has an unusual and very useful "binding isotherm"



Myoglobin:

single chain

hyperbolic binding curve (isotherm)

at pO₂ = 5 mm Hg, 50% saturated

Hemoglobin:

four subunits

sigmoidal binding curve

at pO₂ = 5 mm Hg, 5% saturated

In peripheral tissues, where pO₂ low, hemoglobin easily gives up its ligand

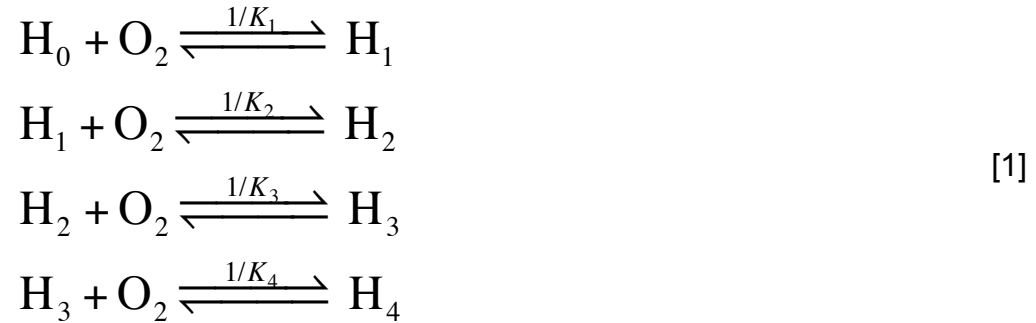
In lungs, where pO₂ high, hemoglobin is easier to saturate than myoglobin

How does hemoglobin accomplish this?

THE ADAIR SCHEME

A simple phenomenological model that is easy to derive but doesn't provide mechanistic insights

The macroscopic binding equilibria:



The macroscopic equilibrium constants:

$$K_j = \frac{[\text{H}_{j-1}][\text{O}_2]}{[\text{H}_j]} \quad [2]$$

Fractional saturation:

$$\bar{y} = \frac{1}{4} \frac{\sum_{i=1}^4 i[\text{H}_i]}{\sum_{i=1}^4 [\text{H}_i]}$$

Expressing each $[\text{H}_i]$ in terms of $[\text{H}_0]$, $[\text{O}_2]$ and K 's, and canceling $[\text{H}_0]$:

$$\bar{y} = \frac{1}{4} \frac{\sum_{i=1}^4 \left(i[\text{O}_2]^i \prod_{j=1}^i 1/K_j \right)}{1 + \sum_{i=1}^4 \left([\text{O}_2]^i \prod_{j=1}^i 1/K_j \right)} \quad [3]$$

Macroscopic binding constants in terms of microscopic binding constants:

$$K_j = \frac{W(n, j-1)}{W(n, j)} k_j \quad [4]$$

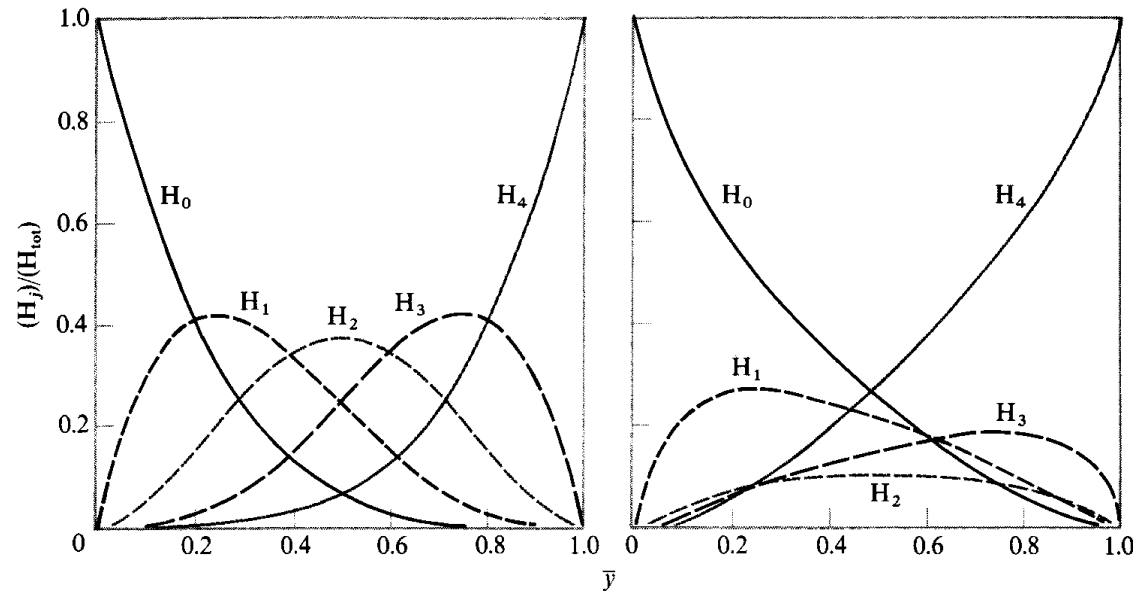
Substituting Eq. 4 into Eq. 3, we get the equation for the binding isotherm:

$$\bar{y} = \frac{[O]/k_1 + 3[O]^2/k_1k_2 + 3[O]^3/k_1k_2k_3 + [O]^4/k_1k_2k_3k_4}{1 + 4[O]/k_1 + 6[O]^2/k_1k_2 + 4[O]^3/k_1k_2k_3 + [O]^4/k_1k_2k_3k_4} \quad [5]$$

Fitting the binding isotherm to Eq. 5 gives:

[NaCl]	k_1	k_2	k_3	k_4
0	8.8	6.1	0.85	0.25
0.1 M	42	13	12	0.14

Using the k_j 's to compute populations $[H_j]$:



COUPLING CONFORMATIONAL EQUILIBRIA TO BINDING: THE MWC MODEL

Assumptions of the Monod-Wyman-Changeux model:

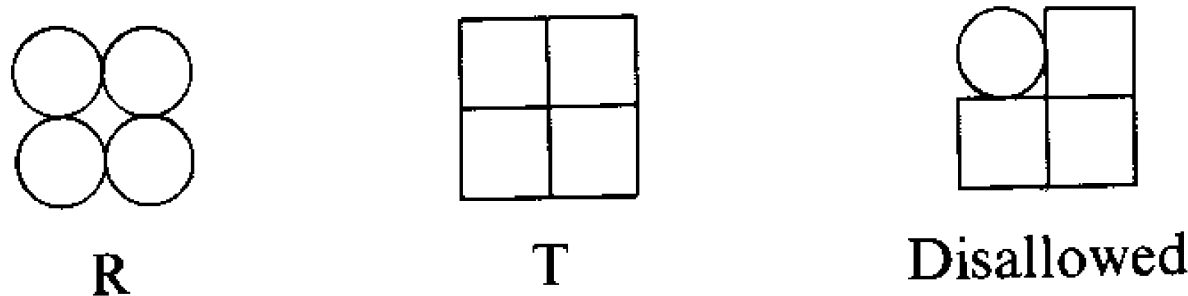
1. Identical protomers (structural unit, may contain more than one polypeptide) occupy equivalent positions in the protein.
2. Each protomer contains a unique receptor site for a specific ligand.
3. At least two conformational state are reversibly accessible and in each, symmetry is conserved.
4. Binding affinity depends only on the conformational state of the enzyme and not the occupancy of neighboring sites.

Homotropic interactions- interactions between identical ligands binding at multiple sites

Heterotropic interactions- interactions between different ligands binding at multiple sites

Algebraic Treatment

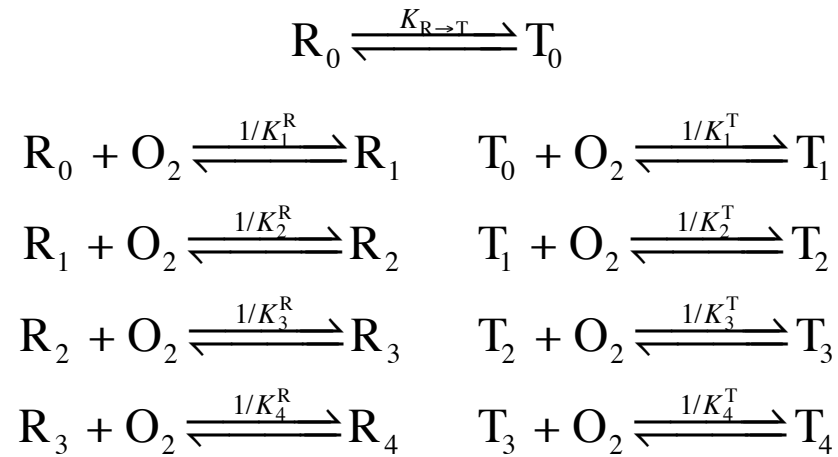
Assumptions require two symmetrical conformational states:



Only two microscopic binding constants needed, k_R and k_T , e.g.:

$$k_T = \frac{\left[\begin{array}{|c|c|} \hline \square & \square \\ \hline \square & \square \\ \hline \end{array} \right] [O]}{\left[\begin{array}{|c|c|} \hline \square & \square \\ \hline \square & \square \\ \hline \end{array} \right]} = \frac{\left[\begin{array}{|c|c|} \hline \square & \text{O} \\ \hline \square & \square \\ \hline \end{array} \right] [O]}{\left[\begin{array}{|c|c|} \hline \square & \text{O} \\ \hline \text{O} & \square \\ \hline \end{array} \right]} = \frac{\left[\begin{array}{|c|c|} \hline \text{O} & \text{O} \\ \hline \text{O} & \square \\ \hline \end{array} \right] [O]}{\left[\begin{array}{|c|c|} \hline \text{O} & \text{O} \\ \hline \text{O} & \text{O} \\ \hline \end{array} \right]} = \dots$$

Macroscopic binding equilibria:



\mathbf{R}_i : Set of all $W(4,i)$ microscopic species of the R conformation that have i \mathbf{O}_2 molecules bound

\mathbf{T}_i : Set of all $W(4,i)$ microscopic species of the T conformation that have i \mathbf{O}_2 molecules bound

$K_{R \rightarrow T}$: Equilibrium constant for the $\mathbf{R}_0 \rightarrow \mathbf{T}_0$ reaction (Cantor & Schimmel call this L)

Macroscopic dissociation constants in terms of the microscopic dissociation constant:

$$\begin{array}{ll}
 K_1^R = \frac{k_R}{4} = \frac{[\mathbf{R}_0][\mathbf{O}_2]}{[\mathbf{R}_1]} & K_1^T = \frac{k_T}{4} = \frac{[\mathbf{T}_0][\mathbf{O}_2]}{[\mathbf{T}_1]} \\
 K_2^R = \frac{2k_R}{3} = \frac{[\mathbf{R}_1][\mathbf{O}_2]}{[\mathbf{R}_2]} & K_2^T = \frac{2k_T}{3} = \frac{[\mathbf{T}_1][\mathbf{O}_2]}{[\mathbf{T}_2]} \\
 K_3^R = \frac{3k_R}{2} = \frac{[\mathbf{R}_2][\mathbf{O}_2]}{[\mathbf{R}_3]} & K_3^T = \frac{3k_T}{2} = \frac{[\mathbf{T}_2][\mathbf{O}_2]}{[\mathbf{T}_3]} \\
 K_4^R = 4k_R = \frac{[\mathbf{R}_3][\mathbf{O}_2]}{[\mathbf{R}_4]} & K_4^T = 4k_T = \frac{[\mathbf{T}_3][\mathbf{O}_2]}{[\mathbf{T}_4]}
 \end{array}$$

[6]

For more compact equations, substitute:

$$\alpha = \frac{[O_2]}{k_R} \quad \text{and} \quad k_T = \frac{k_R}{c} \quad [7]$$

Gives concentration of each R and T species in terms of $[R_0]$, c , α and $K_{R \rightarrow T}$:

$$\begin{aligned} [R_1] &= 4[R_0]\alpha & [T_1] &= 4K_{R \rightarrow T}[R_0]c\alpha \\ [R_2] &= 6[R_0]\alpha^2 & [T_2] &= 6K_{R \rightarrow T}[R_0]c^2\alpha^2 \\ [R_3] &= 4[R_0]\alpha^3 & [T_3] &= 4K_{R \rightarrow T}[R_0]c^3\alpha^3 \\ [R_4] &= [R_0]\alpha^4 & [T_4] &= K_{R \rightarrow T}[R_0]c^4\alpha^4 \end{aligned} \quad [8]$$

Fractional saturation of all forms of hemoglobin with respect to O_2 :

$$\bar{y}_{O_2} = \frac{\sum_{i=0}^4 i[R_i] + \sum_{i=0}^4 i[T_i]}{4 \left(\sum_{i=0}^4 [R_i] + \sum_{i=0}^4 [T_i] \right)} \quad [9]$$

Substituting Eq. [8] into each term of Eq. [9]:

$$\begin{aligned}
 \sum_{i=0}^4 [R_i] &= [R_0](1 + 4\alpha + 6\alpha^2 + 4\alpha^3 + \alpha^4) = [R_0](1 + \alpha)^4 \\
 \sum_{i=0}^4 [T_i] &= K_{R \rightarrow T}[R_0](1 + c\alpha)^4 \\
 \sum_{i=0}^4 i[R_i] &= \alpha \frac{d}{d\alpha} \sum_{i=0}^4 [R_i] = 4[R_0]\alpha(1 + \alpha)^3 \\
 \sum_{i=0}^4 i[T_i] &= 4K_{R \rightarrow T}[R_0]c\alpha(1 + c\alpha)^3
 \end{aligned} \tag{10}$$

Substituting Eq. 10 into Eq. 9:

$$\bar{y}_{O_2} = \frac{4[R_0]\alpha(1 + \alpha)^3 + 4K_{R \rightarrow T}[R_0]c\alpha(1 + c\alpha)^3}{4\left([R_0](1 + \alpha)^4 + K_{R \rightarrow T}[R_0](1 + c\alpha)^4\right)} \tag{11}$$

Eliminate $[R_0]$:

$$\bar{y}_{O_2} = \frac{\alpha(1 + \alpha)^3 + K_{R \rightarrow T}c\alpha(1 + c\alpha)^3}{(1 + \alpha)^4 + K_{R \rightarrow T}(1 + c\alpha)^4} \tag{12}$$

For any n:

$$\bar{y}_{O_2} = \frac{\alpha(1 + \alpha)^{n-1} + K_{R \rightarrow T}c\alpha(1 + c\alpha)^{n-1}}{(1 + \alpha)^n + K_{R \rightarrow T}(1 + c\alpha)^n} \tag{13}$$

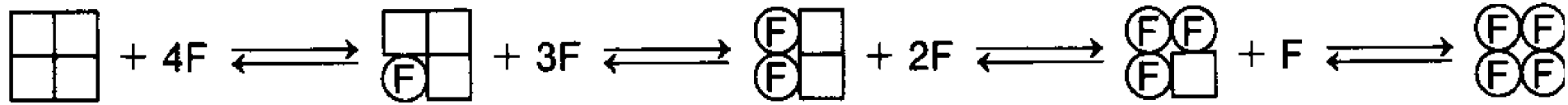
Note that if $K_{R \rightarrow T}=0$ (no taut), then \bar{y}_{O_2} is a hyperbolic saturation curve (no cooperativity):

$$\bar{y}_{O_2} = \frac{\alpha}{(1 + \alpha)}$$

An alternative to MWC: The *sequential model* (KNF-Koshland, Nemethy, and Filmer)

Many of the assumptions of MWC do not apply

The sequential binding/conformational scheme is:



Classic *induced fit* mechanism

Only two subunit conformations: free (square) and bound (circle)

Affinity of each subunit will vary depending on the occupancy of its neighbors

Can explain much more complex data

Usually underdetermined (i.e. too many parameters to be determined uniquely, there are lots of possible solutions)

Combination of MWC & KNF, a more general scheme:

As pointed out by Hammes & Wu (*Science* 172:1205, 1971) other species are possible:

