BioChem 330 - Course Outline

September 27, 2011

- **Bio-molecular Structure/Function (I)**
  - PROTEINS
    - Structure
      - Chemistry of amino acid building blocks
      - Primary, secondary and tertiary structure
      - Protein folding, thermodynamics and kinetics
      - Predictions of protein folding, dynamics

    - Function
      - Binding ….a tale of two globins (hemoglobin and immunoglobulin)

Protein Function – Binding of Ligands

- $\mathbf{A} + B \leftrightarrows C$

- Small Scale: Usually, A is a large macromolecule (protein, enzyme, receptor) and B is a small molecule (ligand, substrate, drug).
  - $P + L \leftrightarrows PL$
  - $E + S \leftrightarrows ES$
  - $R + D \leftrightarrows RD$
- Large Scale: It is also possible for both A and B to be large proteins which is what you see when calmodulin binds to a target protein, a transcription factor binds to DNA, or when an enzyme such as trypsin binds to a protein to initiate digestion.

Protein Function – Binding of Ligands

- In General
  - $A + nB \leftrightarrows C$ $K_a = [C]/[A][B]^n$
  - K_a ↑ = tighter binding
- First consider, n=1
  - Dissociation Eq. Constant K_d
  - $C \leftrightarrows A + B$ $K_d = [A][B]/[C]$
  - K_d inversely related to binding constant, K_a
  - K_d ↑= weaker binding; units of concentration
  - usually $[A_{tot}]$ fixed and small compared to [B]
  - Protein is Saturated with ligand when $[B_{tot}] > K_d$

$$K_a = \frac{[C]}{[A][B]} = \frac{1}{K_d}$$

Protein Function – Binding of Ligands

- $P + L \leftrightarrows P\text{-}L$
- $P_o + L \leftrightarrows P_B$

$$K_a = \frac{[P_b]}{[P_o][L]} = \frac{1}{K_d}$$ Eqn1

- Fractional saturation
  - $[P_T] = [P_B] + [P_o]$
  - $[P_o] = [P_T] - [P_B]$
- Substitute in expression for P_o, get , Eqn 2:

$$\frac{[P_b]}{([P_T]-[P_b])[L]} = \frac{1}{K_d}$$ Eqn 2

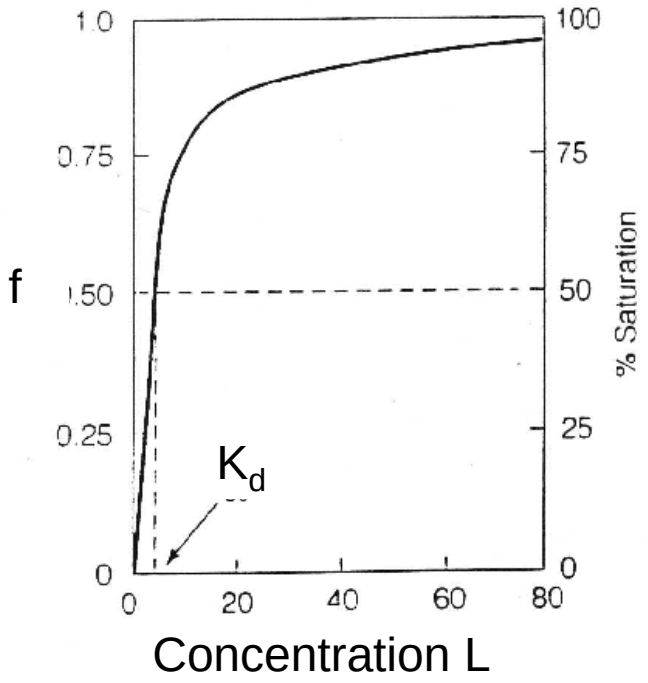
- Simple rearrangement:
  - $f \equiv [P_b]/[P_T]$= saturation

$$f = \frac{[P_b]}{[P_T]} = \frac{[L]}{[L]+K_d}$$ Eqn 3

Protein Function – Binding of Ligands

- Dissociation: $P_b \leftrightarrows P_o + L$
- Dissociation Constant K_d
  - Plot of f vs [L] is hyperbolic
  - When [L] = K_d, f = ½
  - The K_d is equal to the [L] when the protein is half saturated.
  - At conc of [L] above the K_d, protein approaches saturation
  - At conc of [L] below the K_d, the protein in unsaturated.

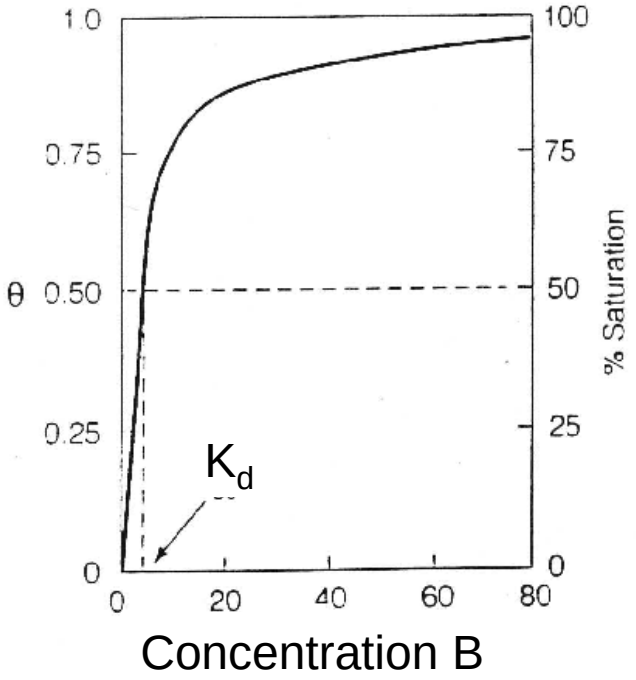
$$f = \frac{[P_b]}{[P_T]} = \frac{[L]}{[L] + K_d}$$



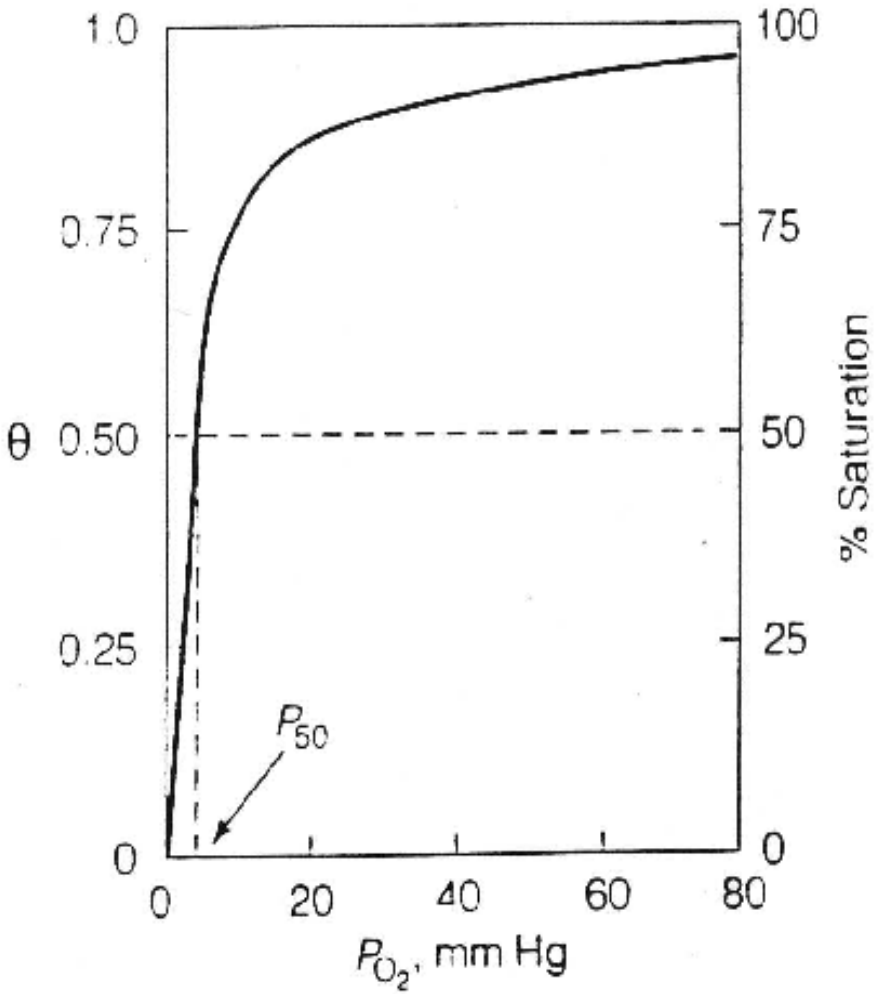
Protein Function – Binding of Ligands

- Dissociation: $P_b \leftrightarrows P_o + L$
- Dissociation Constant K_d
  - Lower K_d tighter the binding
    - Tight binding $K_d < 10^{-9}$ M
      - Example estrogen to ER
    - Medium binding $K_d < 10^{-6}$ M
      - Ca(II) binding to calmodulin
    - Loose binding $K_d < 10^{-3}$ M
      - ATP binding to hexokinase

$$f = \frac{[P_b]}{[P_T]} = \frac{[L]}{[L] + K_d}$$



Protein Function – Binding of Ligands



- At equilibrium
- $Mb\text{-}O_2 \leftrightarrows Mb + O_2$
- $K_d = [Mb]_{free}\,[O_2] / [Mb\text{-}O_2]$
- $K_d = [Mb]_{free}\,pO_2 / [Mb\text{-}O_2]$
- $[Mb]_{free} = [Mb]_T - [Mb\text{-}O_2]$
- Define Y ≡ fractional saturation of Mb
- $Y = [Mb\text{-}O_2] / [Mb]_T$
- Substitute and Rearrange:
  - **$Y = pO_2 / K_d + pO_2$**
  - hyperbola, when Y = ½; $K_d = pO_2$
- Here Y=f and pO_2 = [L]

Binding Curve for O_2 Binding to Mb

Protein Function – Binding of Ligands

- $P_b \leftrightarrows P_o + L$
- Dissociation Constant K_d
  - Lower K_d tighter the binding
    - Tight binding $K_d < 10^{-9}$ M
      - Example estrogen to Receptor
      - *kcal/mole binding energy?*
    - Medium binding $K_d < 10^{-6}$ M
      - Ca(II) binding to calmodulin
      - *kcal/mole binding energy?*
    - Loose binding $K_d < 10^{-3}$ M
      - ATP binding to hexokinase
      - *kcal/mole binding energy?*

Calculate binding energies from K_d using $\Delta G^o = - RT \ln 1/K_d$

Protein Function – Binding of Ligands

- $P_o + L \leftrightarrows P_1 + L \leftrightarrows P_2$

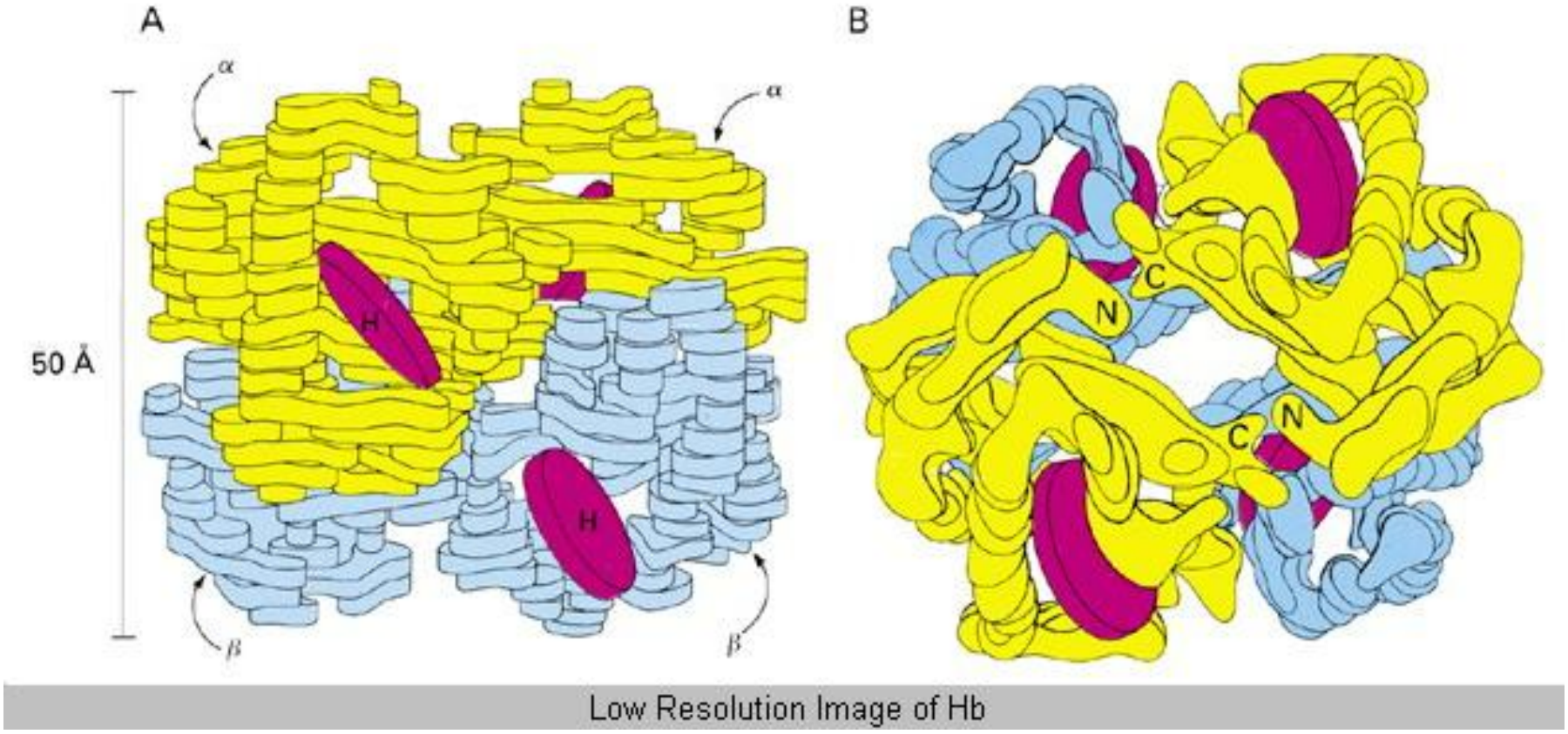
- $\Delta G = \Delta H - T\mathbf{\Delta S}$

  - ΔS an entropic “cost” (decrease in entropy = - **ΔS**) whenever two molecules combine to make one molecule

  - comes from the loss of the translational and rotational movement of two molecules when they become one……..

  - Each molecule has 3 deg of freedom from trans in x, y, and z directions, and 3 deg of freedom from rot about Ix, Iy, and Iz moments of inertia. If this is true, then the two reactant molecules have 12 deg of freedom and the single product molecule has 6, for a net LOSS of 6 deg of freedom.

Hemoglobin Structure: $(\alpha\beta)_2$



Low Resolution Image of Hb

- Hemoglobin is a dimer of dimers, α_1 β_1 dimer α_2 β_2
- see http://www.umass.edu/microbio/chime/hemoglob/2frmcont.htm

Hemoglobin – Elaine Lin

PDB code of structure: 1HHO

Name each molecule in complex:

4 subunits each with 1 heme prosthetic group (which itself has a Fe2+ atom), 1 oxygen for each subunit (when bounded)

Source of information about function and structure:

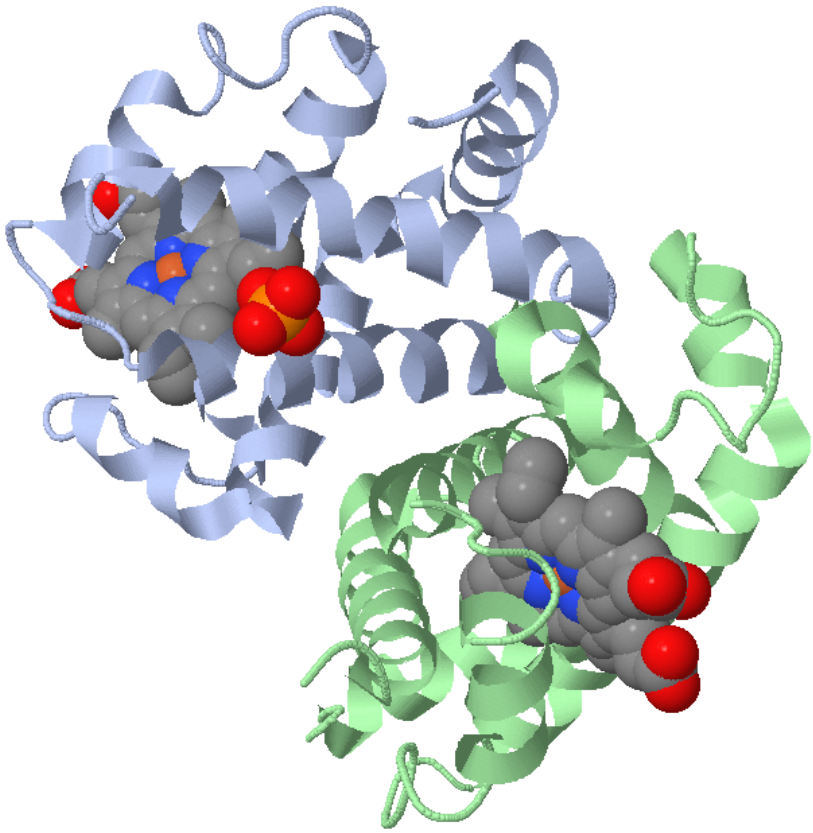
http://www.pdb.org/pdb/101/motm.do?momID=41

http://proteopedia.org/wiki/index.php/Hemoglobin

Voet, Fundamentals of Biochemistry: 181-196

Functionally relevant residues and features of the structure you will explore:

- Differences in conformation between oxygen bound and no oxygen bound (residue involved: histidine)
- Sickle cell hemoglobin: glutamate 6 in the beta chain mutated to valine
- Fetal hemoglobin versus maternal hemoglobin

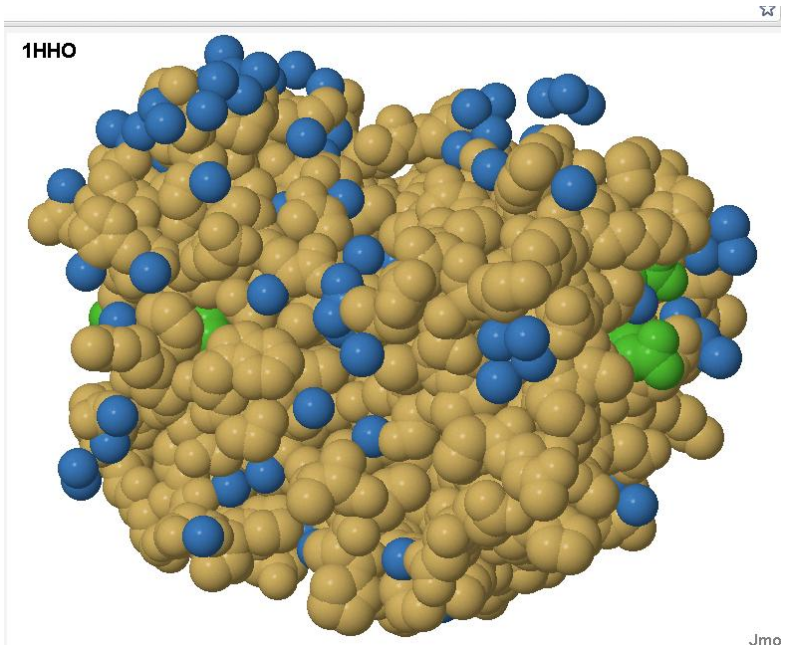


Knobby Surface View

Composition: All atoms are shown as spacefilling spheres of van der Waals radii, colored as follows:

- Protein
- DNA
- RNA
- "Ligand"*
- Solvent**

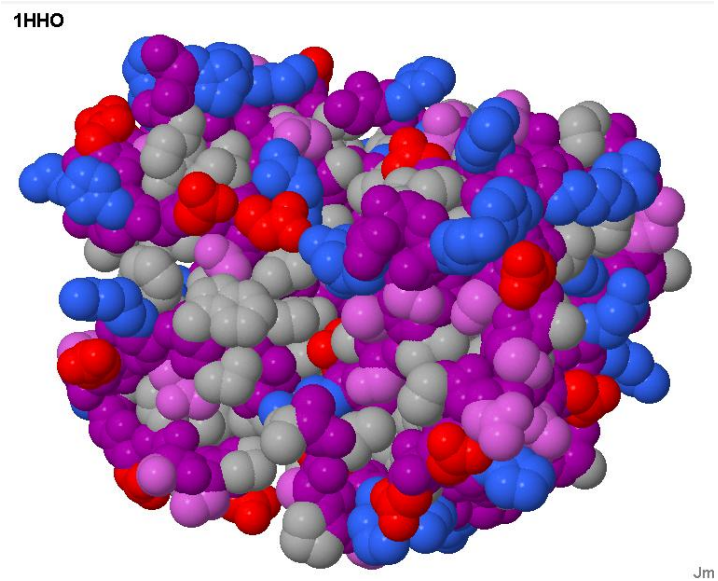
* Here, *"Ligand"* includes everything that is not protein, DNA, RNA, or solvent.
***Solvent* is water plus inorganic sulfate or phosphate ions.**



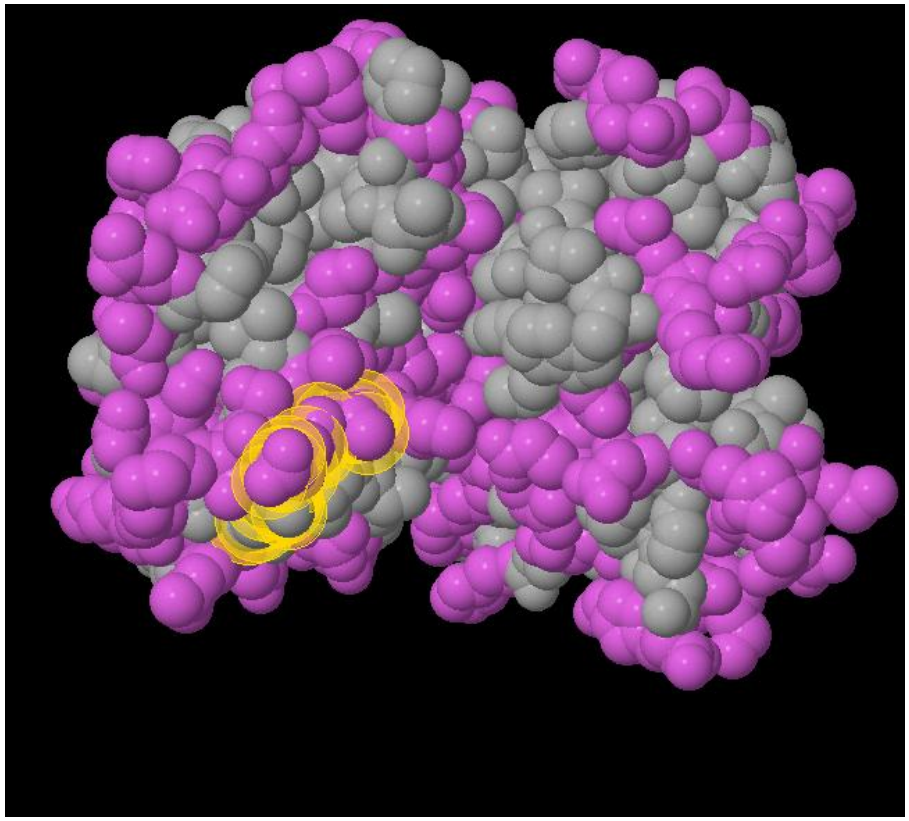
Charged Surface

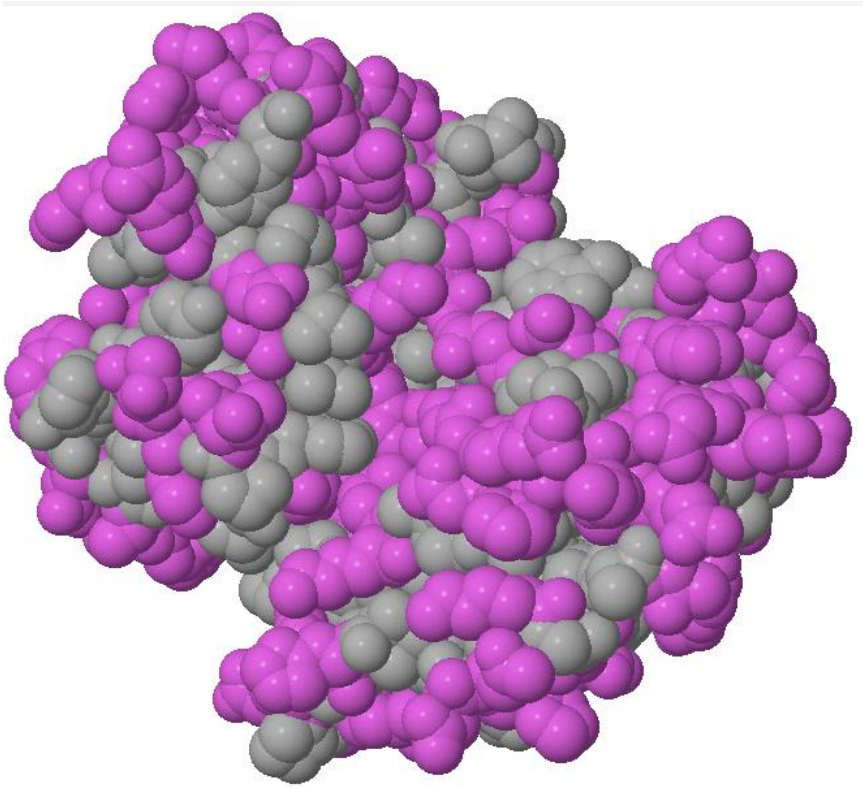
Charge: Amino acids are colored

- Cationic + (entire sidechains)
- Anionic - (entire sidechains)
- Polar, uncharged
- Backbone atoms (charged or uncharged)
- Hydrophobic
  - ☑ Color polar (uncharged) sidechains.



Hydrophobic/Polar

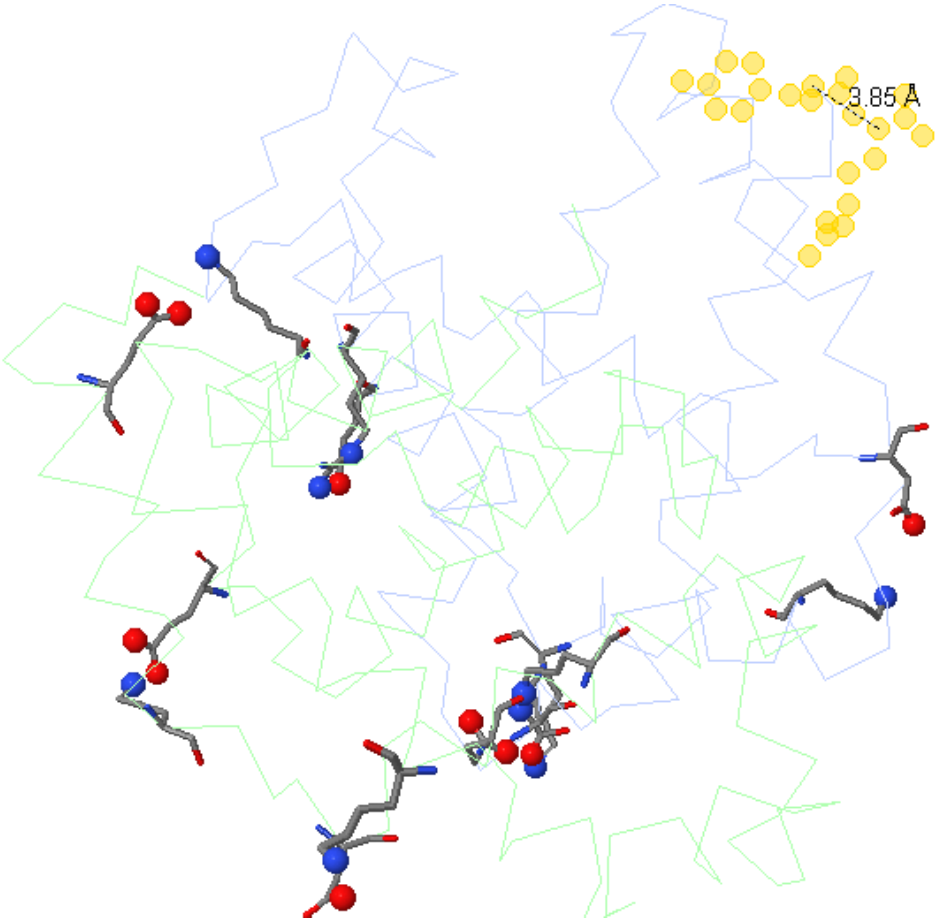




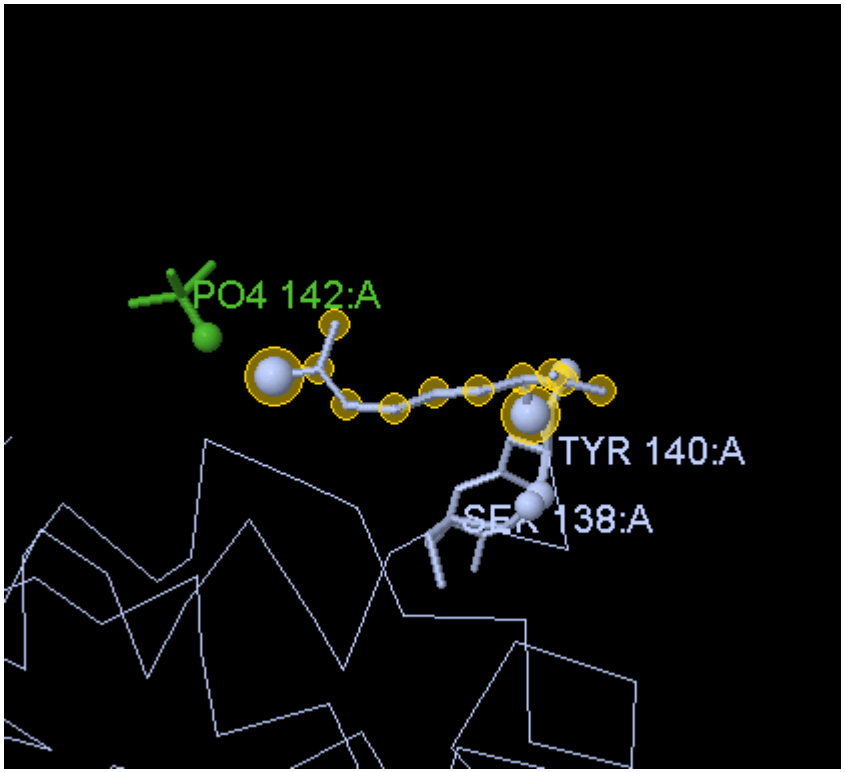
Tyr 140 Chain = A Element = C Atom = C
Arg 141 Chain = A Element = C Atom = C

Salt Bridges + Cation Pi interactions + Core Residue Distance

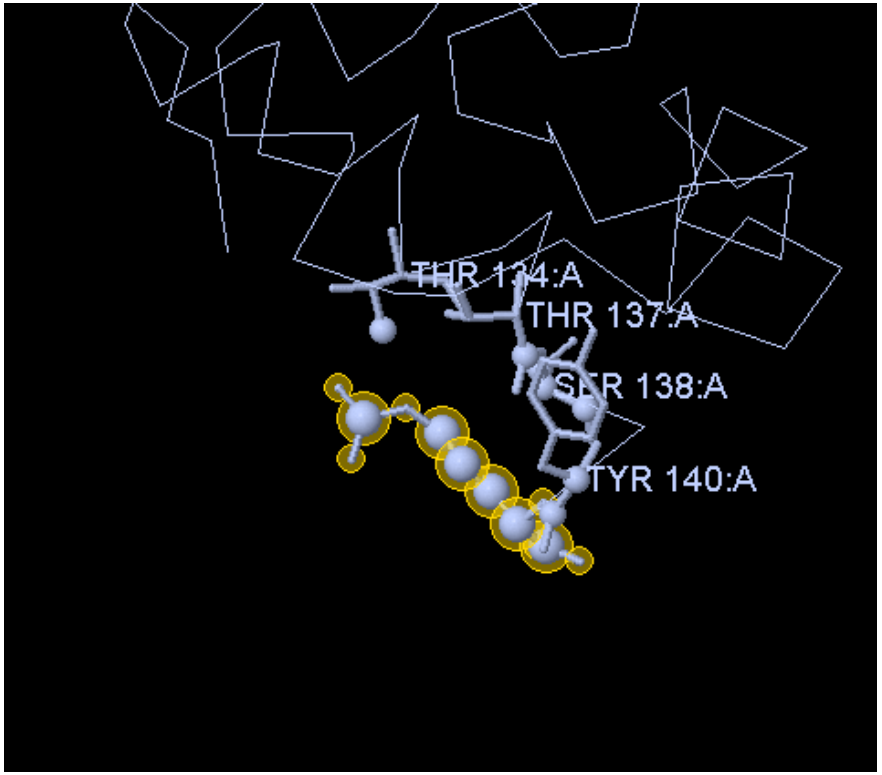
- 3.85 A between C-alpha's of Try 140 and Arg 141
- Salt Bridges
  - Glu 7-Lys 132
  - Glu 27-Arg31
  - Asp 74-Lys 7
  - Glu 116-Lys 16
  - Glu 26-Arg 30
  - Glu 121-Lys 17
  - Asp 21-Lys 65
- Cation Pi:
  - Arg 40-Phe 41 (only one)



Arg 141 Contacts



H Bonds



Hydrophobic

1HHO structural information

- Humans, 2.1 A
- No expression system
- By homologous chain: 1HGB, 1JEB, 1M9P

Arg 141 Contacts in CSU

Table I

Solvent accessible surface (Å^2) for ARG 141 (chain A) in PDB entry 1HHO .

Legend:
In order to see to what extent the residue is buried, accessible surfaces are given for two cases: in the protein and "free" (in a vacuum).

Atom	In protein	Free
N	0.0	46.1
CA	9.9	17.5
C	13.2	19.3
O	28.9	39.2
CB	12.1	19.3
CG	9.4	21.3
CD	10.3	33.9
NE	0.2	18.0
CZ	7.9	12.1
NH1	46.5	50.3
NH2	10.1	58.6
OXT	34.5	35.9
TOTAL	183.0	371.4

Table II

Residues in contact with ARG 141 (chain A) in PDB entry 1HHO

Legend:
Dist - nearest distance (Å) between atoms of two residues
Surf - contact surface area (Å^2) between two residues
HB - hydrophilic-hydrophilic contact (hydrogen bond)
Arom - aromatic-aromatic contact
Phob - hydrophobic-hydrophobic contact
DC - hydrophobic-hydrophilic contact (destabilizing contact)
+/- - indicates presence/absence of a specific contacts
* - indicates residues forming contacts by their side chain (including CA atoms)

Residue		Dist	Surf	Specific contacts			
				HB	Arom	Phob	DC
134A	THR*	3.7	37.5	+	-	-	+
137A	THR*	3.5	21.9	+	-	-	+
138A	SER*	3.6	35.5	-	-	-	+
139A	LYS	3.3	4.6	-	-	-	+
140A	TYR*	1.3	84.1	-	-	+	+
142A	PO4	3.3	28.1	+	-	-	-

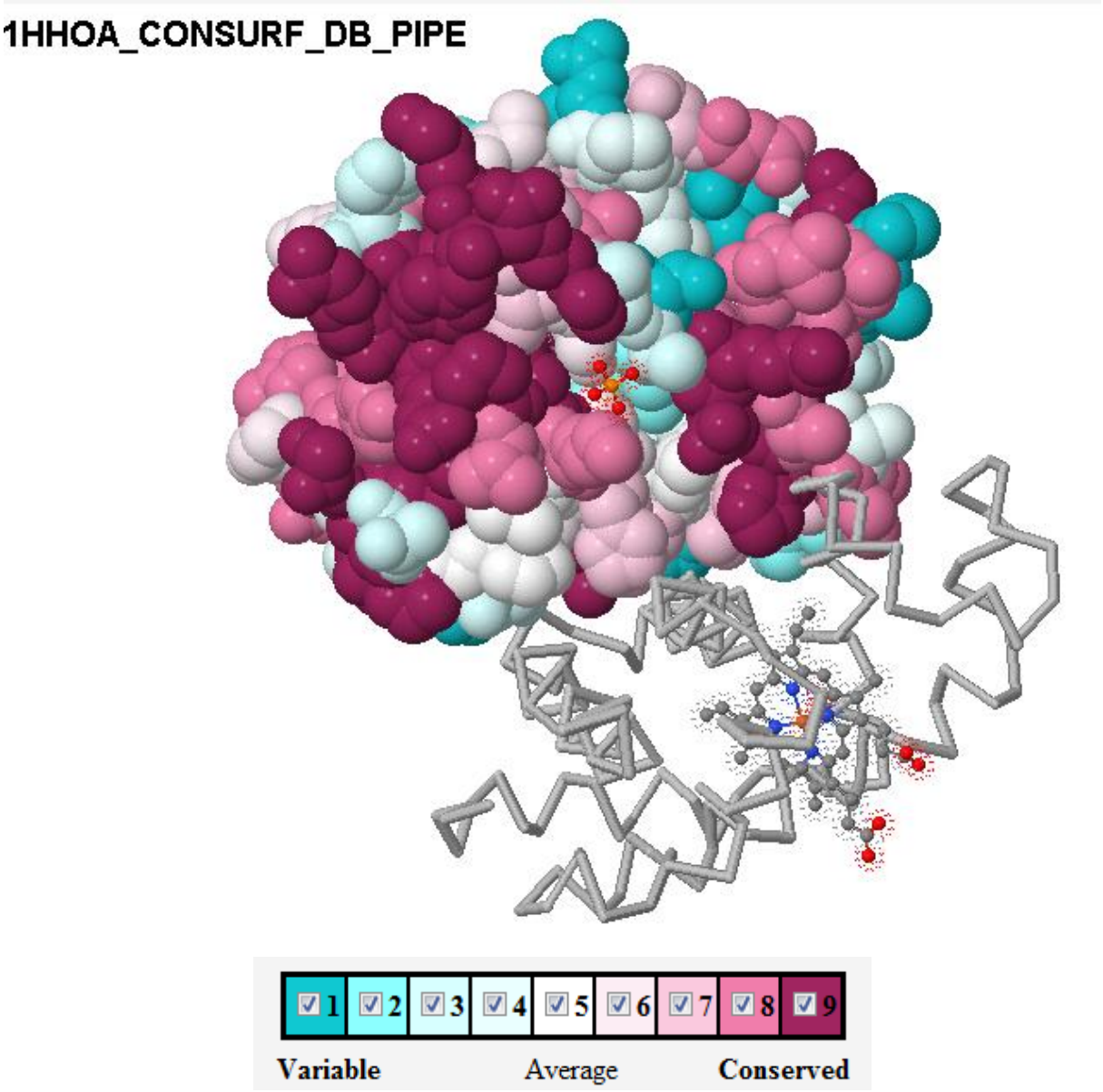
Table III

List of putative hydrogen bonds formed by ARG 141 (chain A)

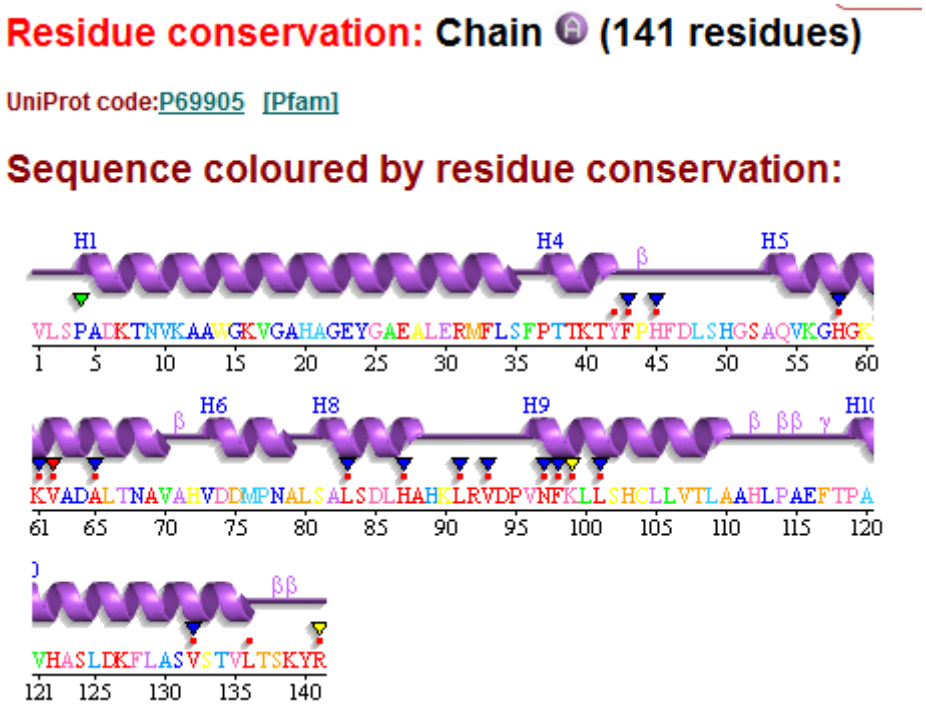
Legend:
Dist - distance (Å) between the atoms
Surf - contact surface area (Å^2) between the atoms

Atom from ARG 141		Contacting atom			Dist	Surf
Name	Class	Residue	Name	Class		
NE	III	THR 137A	OG1	I	3.8	9.5
NE	III	THR 137A	O	II	4.2	0.2
NH1	III	PO4 142A	O1	I	4.6	2.0
NH1	III	PO4 142A	O3	I	5.3	1.6
NH1	III	PO4 142A	O4	I	5.7	0.2
NH2	III	PO4 142A	O1	I	3.3	21.8
NH2	III	THR 134A	OG1	I	3.7	5.9
NH2	III	THR 137A	OG1	I	4.5	2.0
NH2	III	PO4 142A	O3	I	4.5	2.4

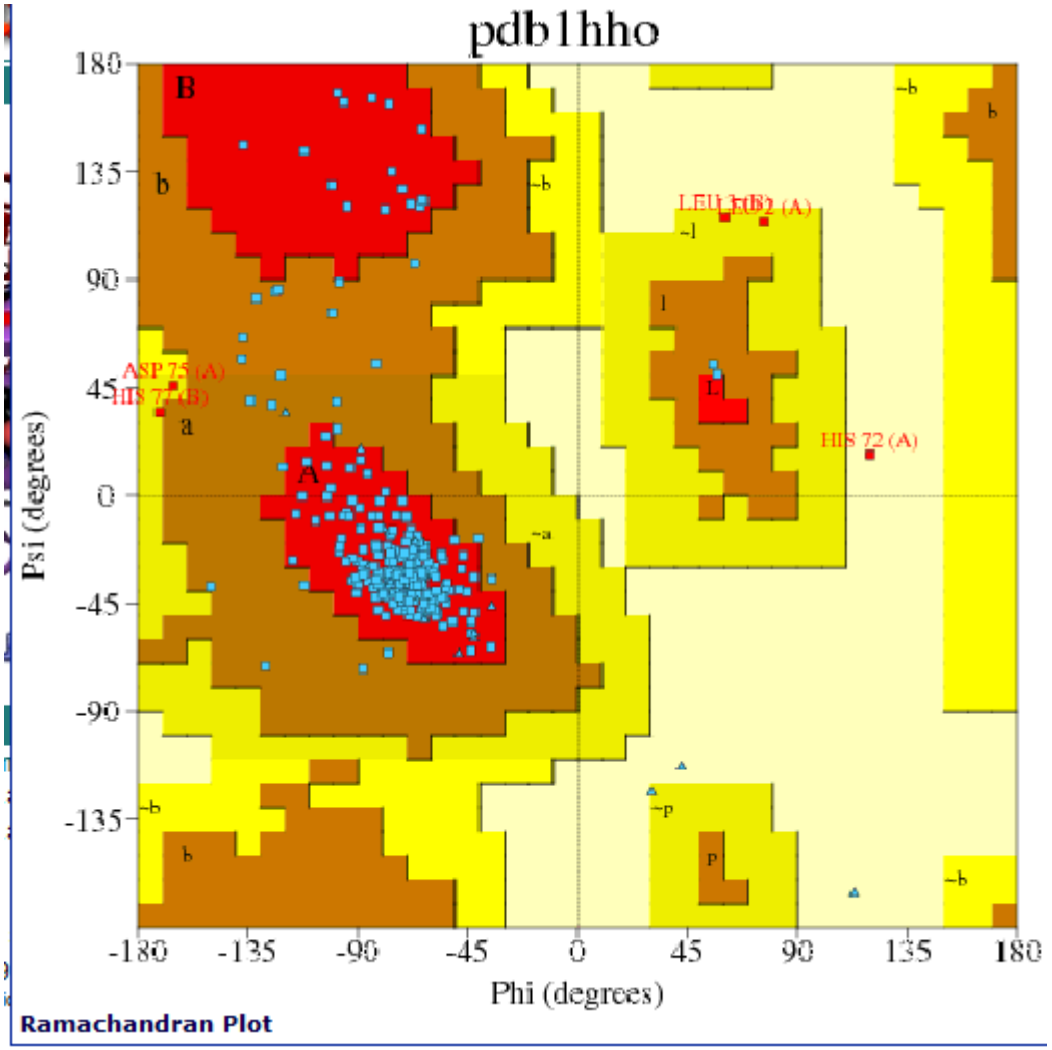
Conservation



Residue Conservation



1HHO Ramachandran plot



Oxygen Binding

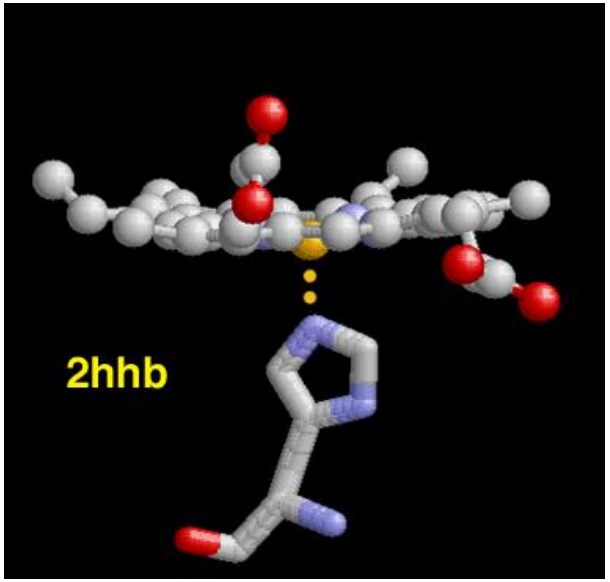
Oxygen binding is cooperative.
Oxygen binding changes the conformation of entire hemoglobin tetramer from T to R.

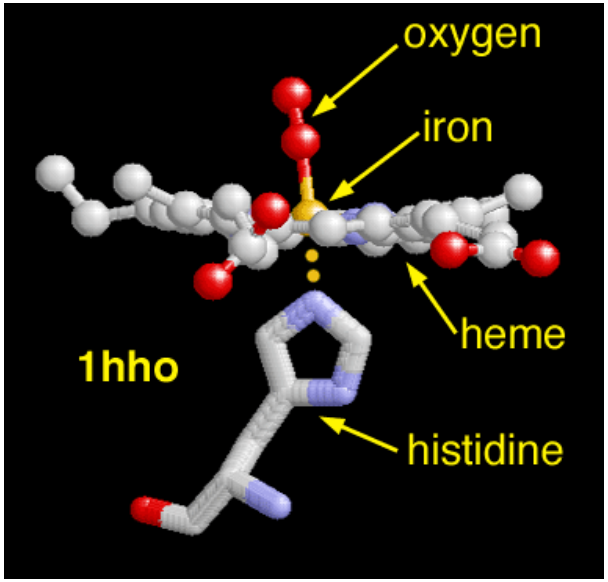
Oxygen binds to the iron of the heme group of hemoglobin subunit.

Consequently, the oxygen pulls up the iron which then also pulls up the histidine it has bonded to.

This shift causes conformational changes in the single subunit, which then initiates changes in the other subunits to increase binding affinity. (This mechanism produces cooperative binding.)

http://www.pdb.org/pdb/101/motm.do?momID=41





Sickle Cell Hemoglobin

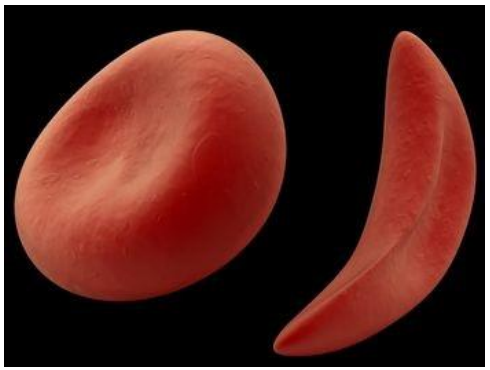
Mutation: Val replaces Glu at 6^{th} position on each beta chain

Val fits into hydrophobic pocket of the beta subunit of another hemoglobin tetramer, forming linear polymers.

Blood cells become sickle-shaped (elongated) and cannot travel through capillaries, resulting in tissue death if blood cannot reach.

Sickle cell anemia (hemoglobin S) protects against malaria: sickle cells break down and are removed by the spleen more readily when infected with Malaria parasite

Voet, 195-197



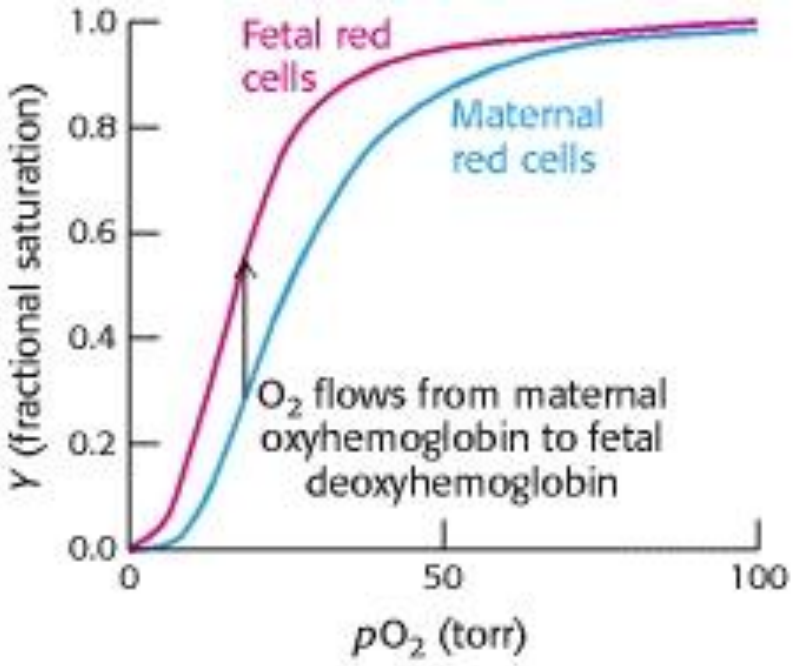
Fetal Hemoglobin

Fetal hemoglobin has higher affinity for oxygen than maternal hemoglobin, making transfer of oxygen from mother's blood more successful.

Conformation: fetal hemoglobin has gamma chains instead of beta and instead of His 143 (cation), has Ser 143 (uncharged)

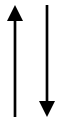
His usually stablizes deoxyhemoglobin, so if replaced by Ser, infant hemogloblin destabilizes deoxyhemoglobin, thereby stabilizing oxyhemoglobin.

http://www.ncbi.nlm.nih.gov/books/NBK22596/



Hemoglobin Structure: O_2 Binding

Oxy R state relaxed



Deoxy T state tense

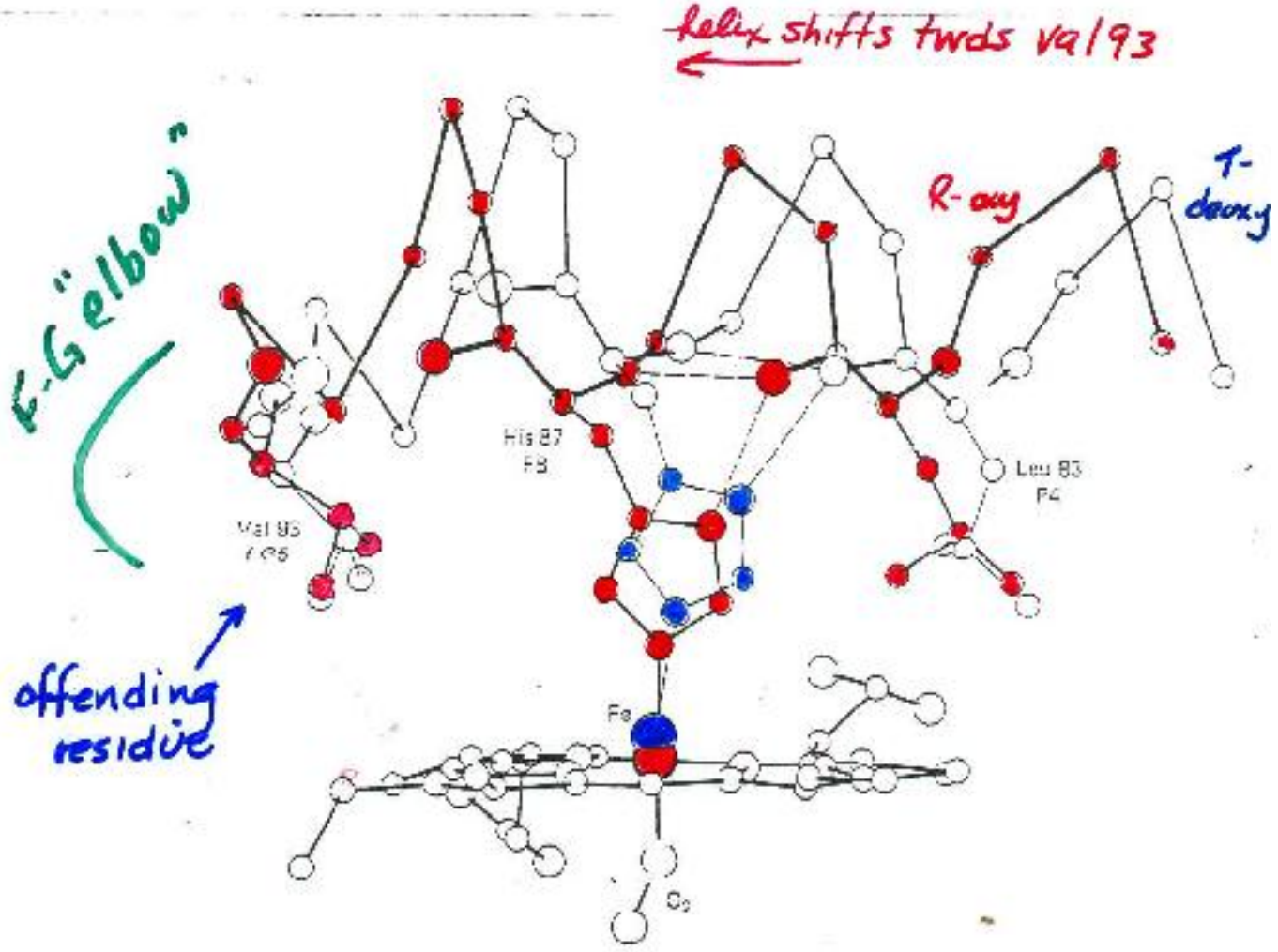
	Name of contact	Waals contacts	Hydrogen bonds	Salt bridges
Horse hemoglobin				
oxy (321)	$\alpha_1\beta_1$	110	5	0
	$\alpha_1\beta_2$	80	1	0
	$\alpha_1\alpha_2$	0	0	0
	$\beta_1\beta_2$	0	0	0
deoxy (322)	$\alpha_1\beta_1$	98	5	0
	$\alpha_1\beta_2$	69	1	1
	$\alpha_1\alpha_2$	0	0	2
	$\beta_1\beta_2$	0	0	0

Hb + $4O_2 \rightleftharpoons$ **Hb(O_2)$_4$**

**** Do these data support the statement that Hb is a dimer of dimers?***

**** What about the structural change from deoxy to oxy?***

Hb: Changes at Heme upon O_2 Binding



- **T state Lighter** lines are deoxy Hb, Fe out of plane
- **R state Bold** Lines are oxy Hb, Fe in plane of Heme

Hb + $4O_2 \rightleftharpoons$ **Hb**$(O_2)_4$

Protein Function

Cooperative Binding of Ligands

- examine 2 ligands binding in concert (binding of 1st ligand immediately followed by binding of 2nd ligand)
  - $P_o + L \leftrightarrows P_1$ $K_{d1} = [P_o][L]/[P_1]$ first ligand
  - $P_1 + L \leftrightarrows P_2$ $K_{d2} = [P_1][L]/[P_2]$ second ligand
- Net reaction:
  - $P_o + 2L \rightleftarrows P_2$ $K_{d1}K_{d2} = P_o[L]^2/P_2$
  - $P_o = (1\text{-}Y)(P_T)$
  - Plot of ln(Y/1-Y) vs ln[L] will give
  - slope of n, the number of sites

$$K_1K_2 = \frac{[P_o][L]^2}{P_2}$$

$$K_1K_2P_2 = [(1-Y)P_T][L]^2$$

$$\frac{P_2}{[(1-Y)P_T} = \frac{[L]^2}{K_1K_2}$$

$$\frac{Y}{1-Y} = \frac{[L]^2}{K_1K_2}$$

$$\ln\left(\frac{Y}{1-Y}\right) = 2\ln[L] - \ln(K_1K_2)$$

Protein Function

Cooperative Binding of Ligands

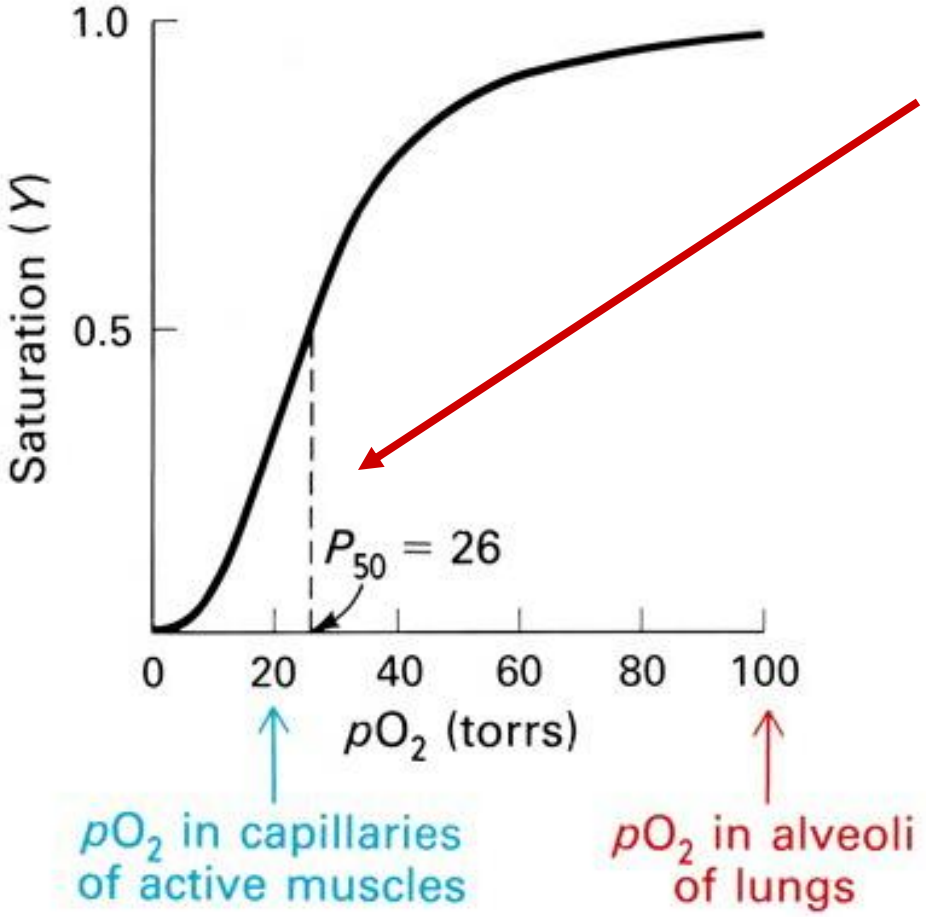
- Now, consider a protein with 4 cooperative sites
- $P_o + L \leftrightarrows P_1$ $K_{1d} = P_o[L]/P_1$
- $P_1 + L \leftrightarrows P_2$ $K_{2d} = P_1[L]/P_2$
- ……
- $P_3 + L \leftrightarrows PL_4$ $K_{4d} = P_3[L]/P_4$
- Again, above are macroscopic dissociation constants…….

At limit, perfect cooperativity, system doesn't exist in intermediate states

$K_{doverall} = P_o[L]^4/P_4$

- How many individual microstates exist?
- 4 infinitely **cooperative sites**, a,b,c,d:

Protein Function
Cooperative Binding of Ligands



How Hb transports oxygen

Hb has four binding sites

Unlike Mb or Tf, L binding to Hb is **not** hyperbolic but sigmoidal

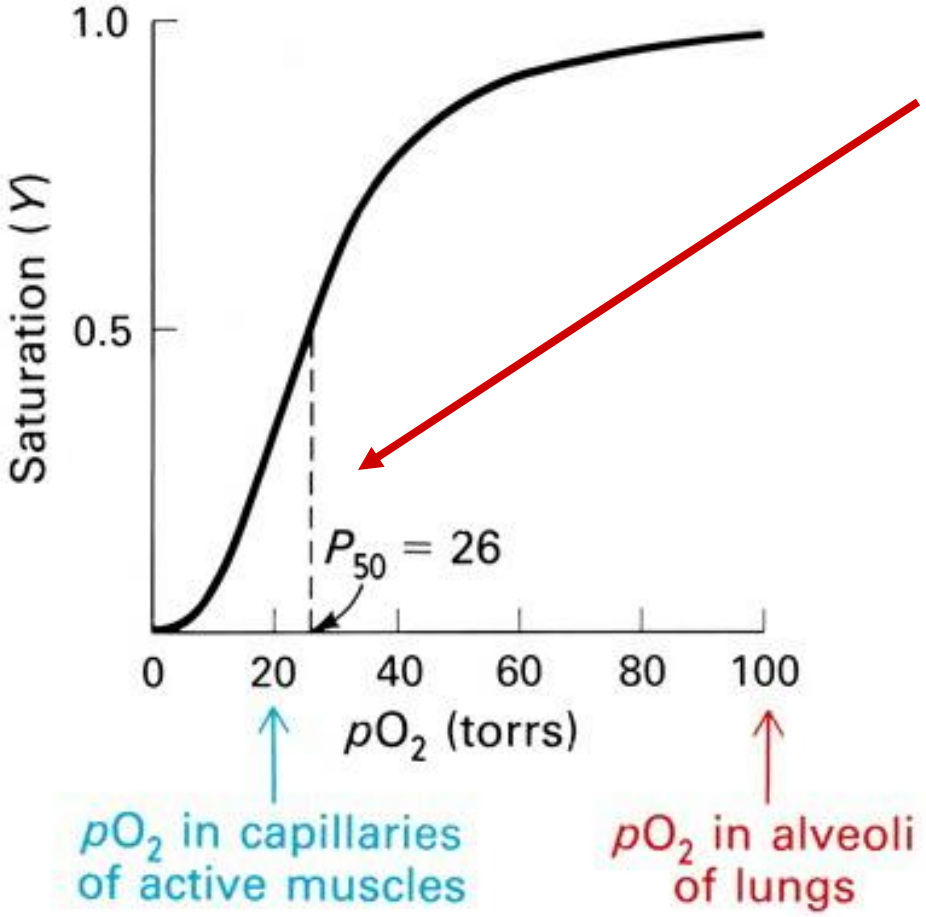
binding of first ligand increases the affinity of the remaining sites for ligand

Examine concerted model:

$$\mathbf{Hb} + 4O_2 \rightleftharpoons \mathbf{Hb(O_2)_4} \quad K_a$$

$$\mathbf{Hb(O_2)_4} \rightleftharpoons \mathbf{Hb} + 4O_2 \quad K_d$$

Protein Function
Cooperative Binding of Ligands



How Hb transports oxygen

O_2 binding is cooperative

Hb + $4O_2$ ⇌ **$Hb(O_2)_4$** K_a

$Hb(O_2)_4$ ⇌ **Hb** + $4O_2$ K_d

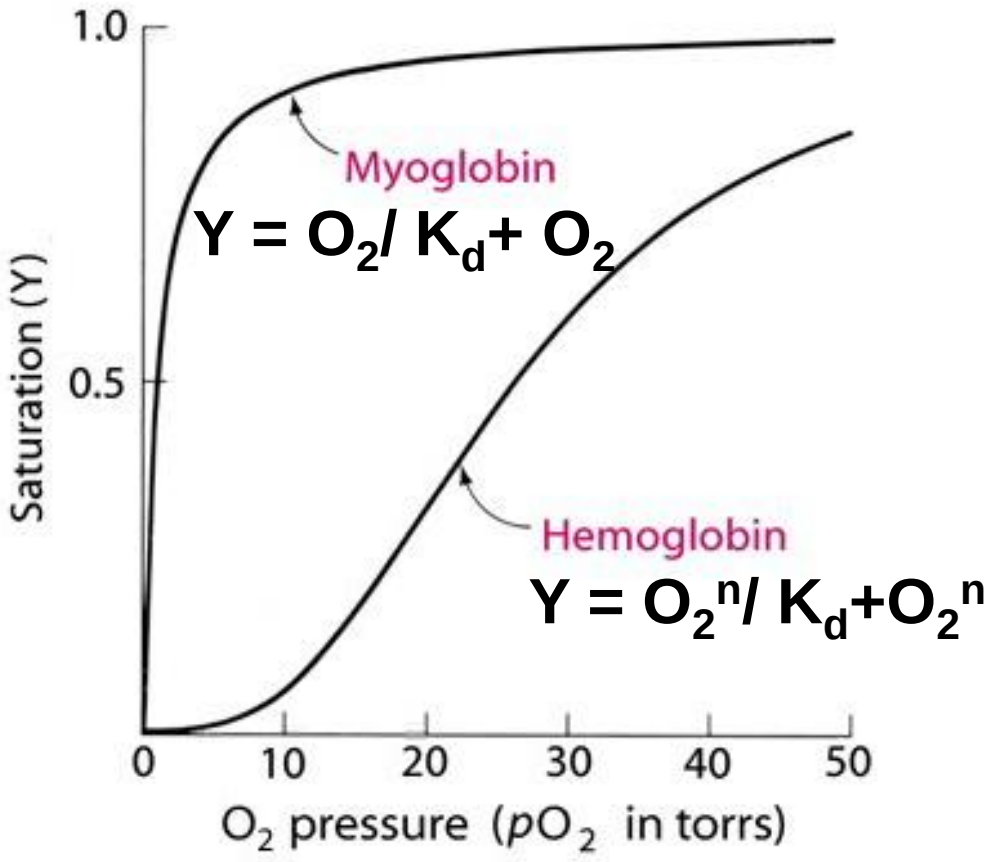
$K_d = [Hb][O_2]^4/[Hb(O_2)_4]$

$Y = [Hb(O_2)_4]/[Hb_T]$

$Y = pO_2^n / K_d + pO_2^n$

if perfect cooperativity, n = 4

Protein Function
Cooperative Binding of Ligands



Saturation Curves for Mb and Hb

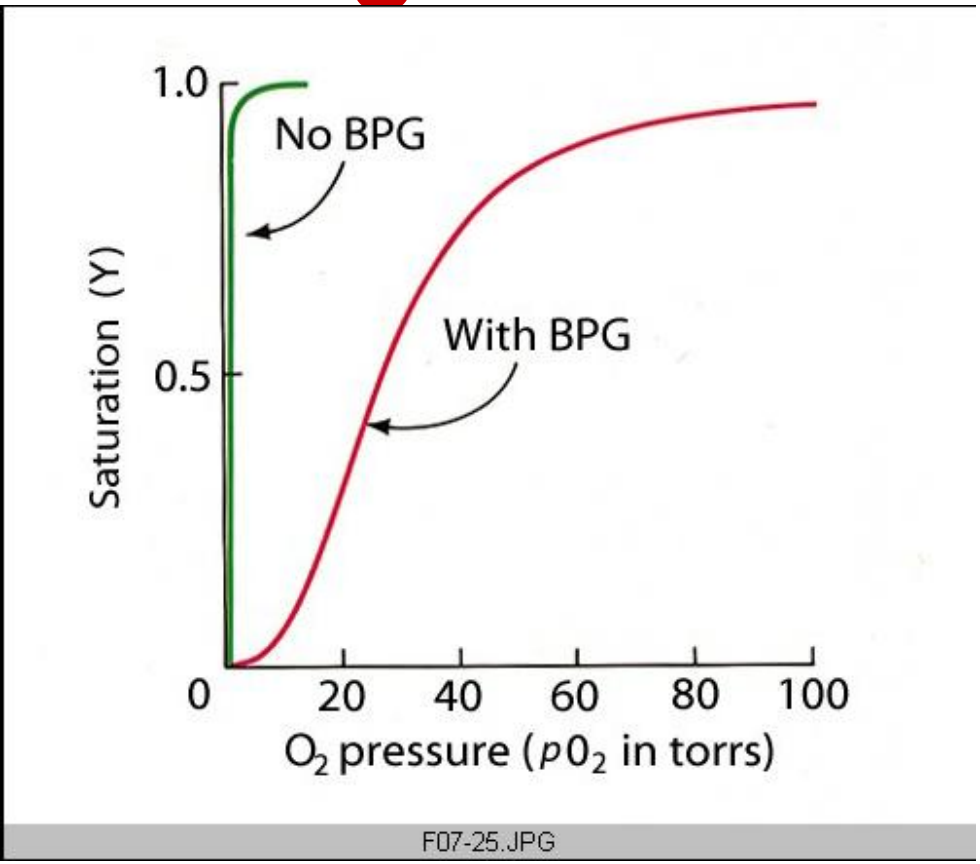
* Hb changes its affinity for oxygen as oxygen pressure increases.

* oxygen binding curve is sigmoidal.

* tetramer is cooperative

* Cooperativity: tetramer behaves differently than monomer. K_a, K_d change as oxygen level increases

Hemoglobin Structure: BPG Binding



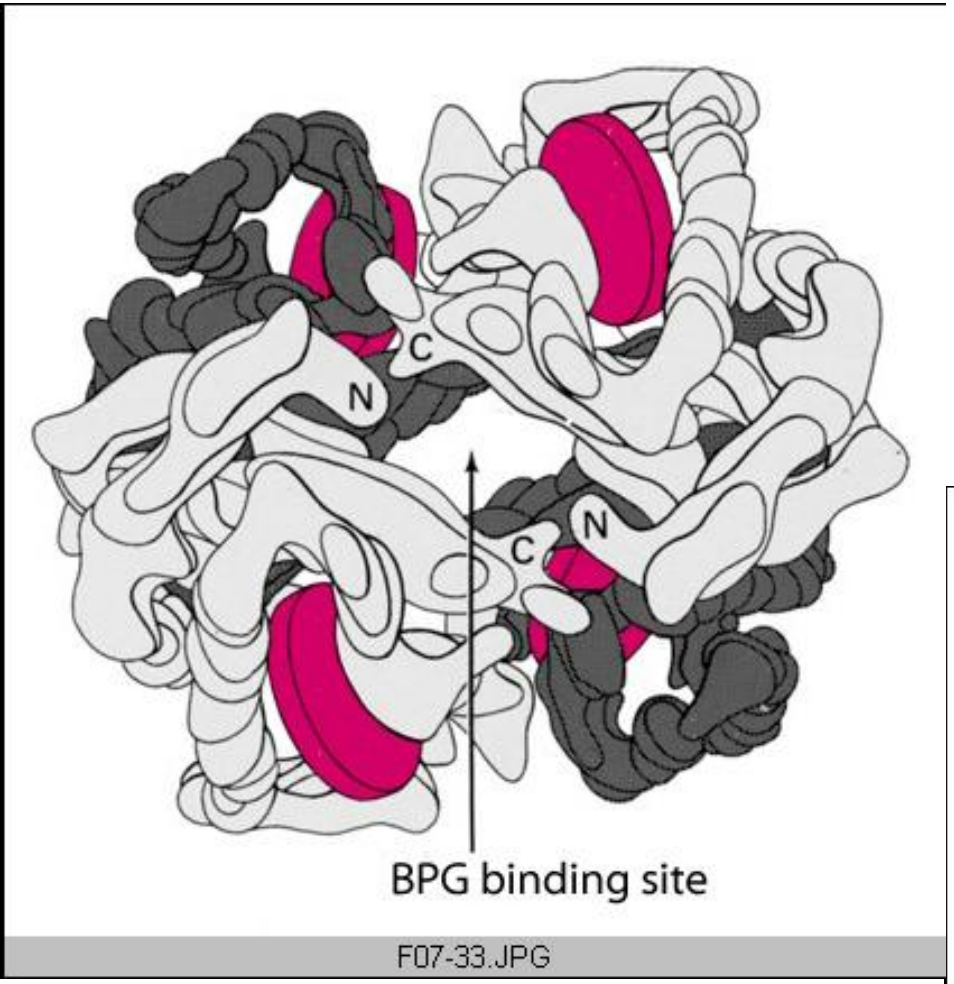
*__Allosteric Regulators__ are molecules or atoms which affect the function of a protein from afar, that is they do not bind at the active site, but elsewhere on the protein.

* BPG has been known to stabilize the deoxy state for about 80 years.

* without BPG,

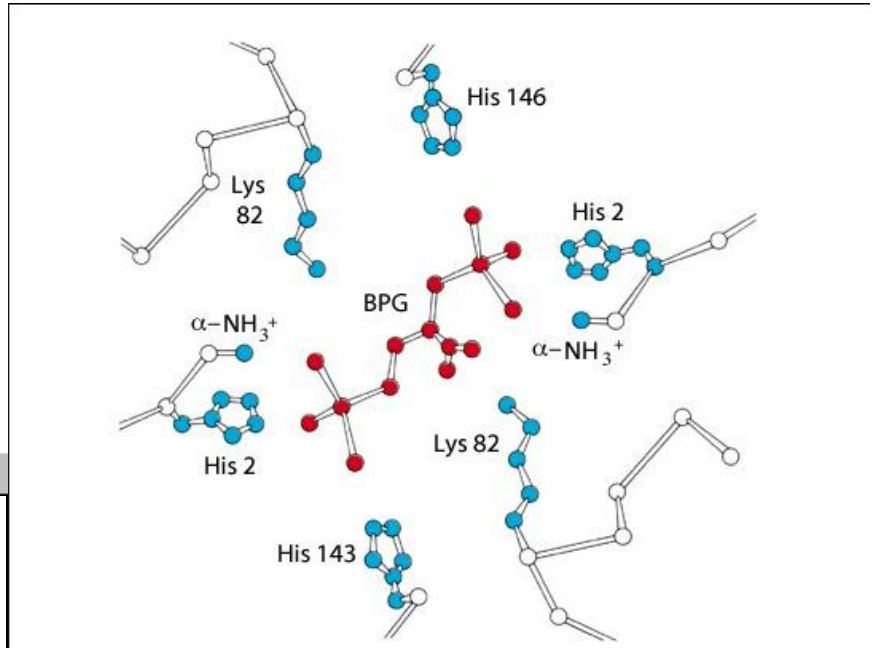
Hb looks just like Mb

Hemoglobin Structure: BPG Binding



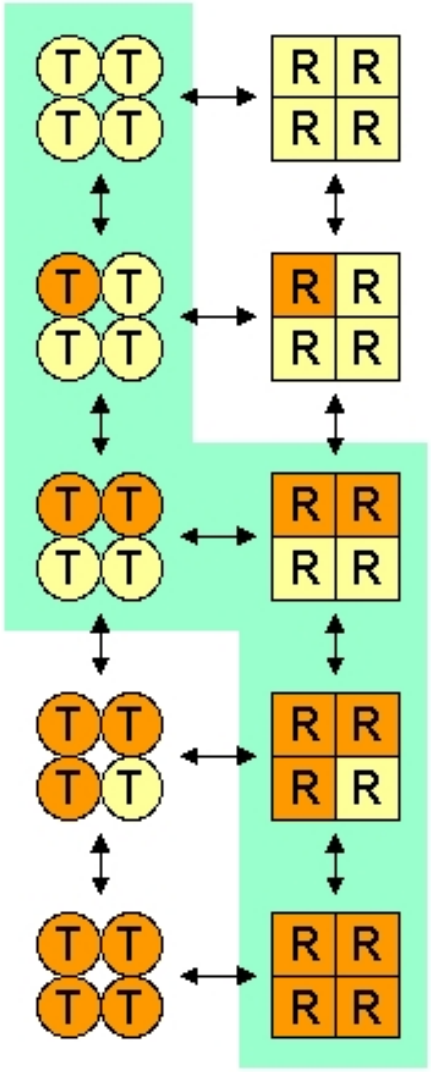
F07-33.JPG

BPG binds to three positive residues on each β–chain



F07-34.JPG

Molecular Associations: cooperativity



Monod Wyman Changeux: Symmetry model, concerted model, all or nothing model.

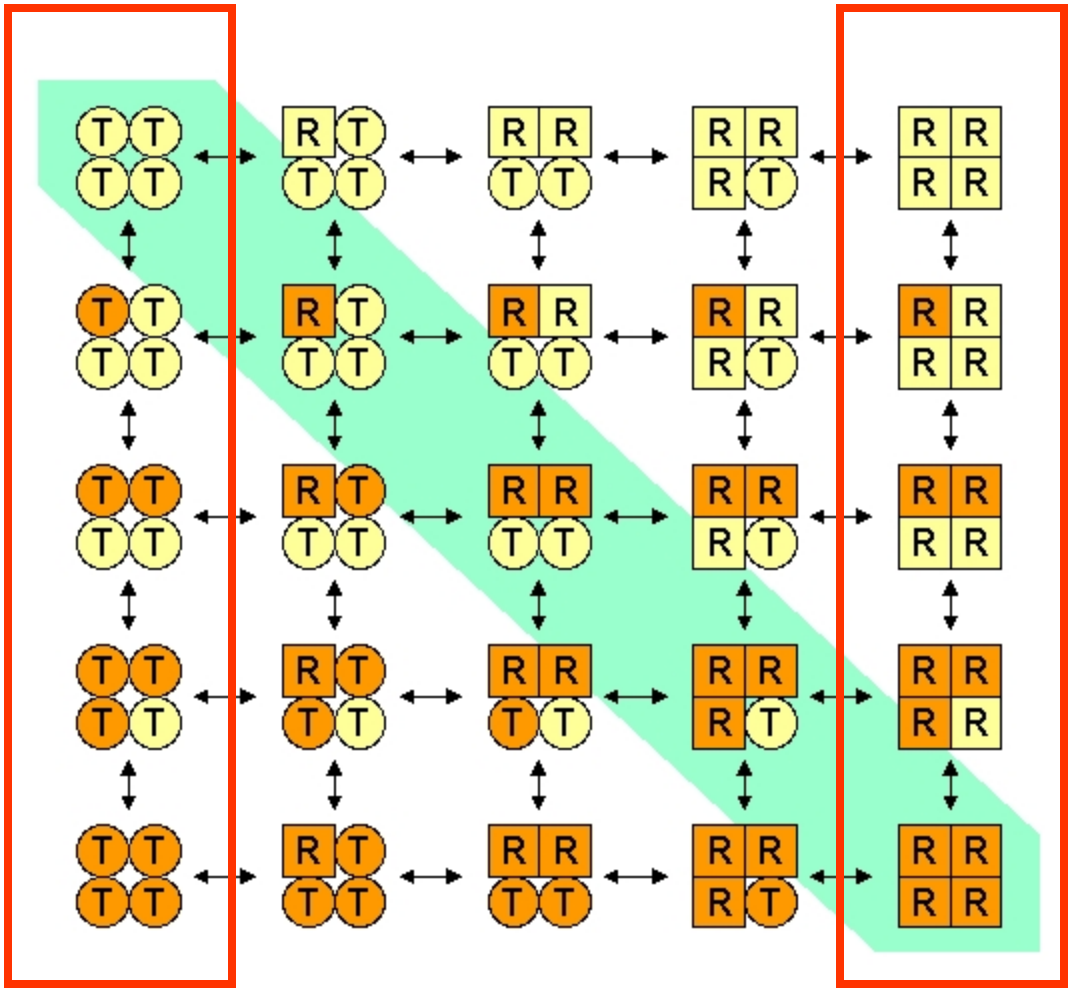
Binding of one ligand changes the entire tetramer to a high affinity conformation.

Binding events (up and down) and conformational transitions (left and right)

This model needs five equilibria for conformations and eight equilbria for binding events. (13 constants to deal with)

Dominant species are tracked by the blue shading, showing low affinity T site at low Oxygen tension and a switch over somewhere between one and two O2 binding to the ALL R state which has high affinity.

Molecular Associations: cooperativity



Sequential Model is another model which says that individual subunits may have different conformations, changes are not happening together.

Binding equlibria are represented on horizontal axis and binding events occur on the vertical axis

Need 20 different conformational equilbria (4x5) and 20 different binding constants (5x4) to fully describe the system

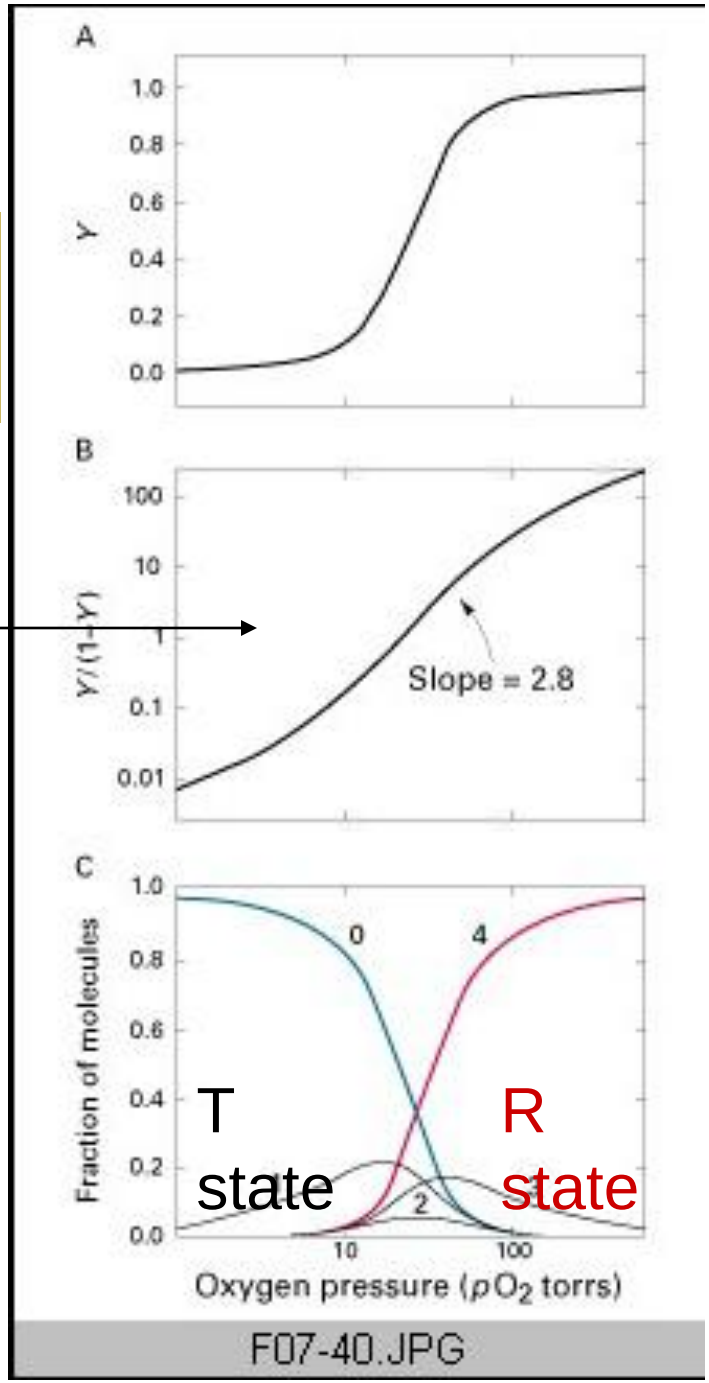
Two columns at ends represent Symmetry model

Cooperativity

Hill Equation:

Log [Y/(1-Y)] = n_H log[L] - log K_d

- Hill Plot: log[Y/(1-Y)] vs log[L]
- Hill Coefficient: n_H, slope of the linear portion of the Hill Plot that crosses y=0 (half saturated)
- $n_H \leq n$ (the actual number of sites)
- closer n_H is to n, the more cooperative the system
- K_d can be determined from Y intercept (–log $K_{doverall}$)



Protein Function

Cooperative Binding of Ligands

- FROM BEFORE 2 Ligands bind together (Binding of 1 ligand immediately followed by binding of second ligand)
- $P_o + L \leftrightarrows P_1$ $K_1 = [P_o][L]/[P_1]$ binding of first ligand
- $P_1 + L \leftrightarrows P_2$ $K_2 = [P_1][L]/[P_2]$ binding of second ligand

- NOW General Reaction:
  - $P_o + nL \leftrightarrows PL_n$ $K_1K_2\ldots\ldots K_n = P_o[L]^n/PL_n$
  - $P_o = (1\text{-}Y)(P_T)$ $K_{doverall} = K_1K_2\ldots\ldots K_n$

$$K_1K_2....K_n = \frac{[P_o][L]^n}{Pn}$$

$$K_{doverall}P_n = [(1-y)P_T][L]^n$$

$$\frac{P_n}{[(1-y)P_T} = \frac{[L]^n}{K_{dn}}$$

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

$$\ln\left(\frac{Y}{1-Y}\right) = n\ln[L] - \ln(K_d)$$

Hill Plot

- Plot of ln(Y/1-Y) vs ln[L] will give
- slope of n, the HILL COEFFICIENT

Protein Function

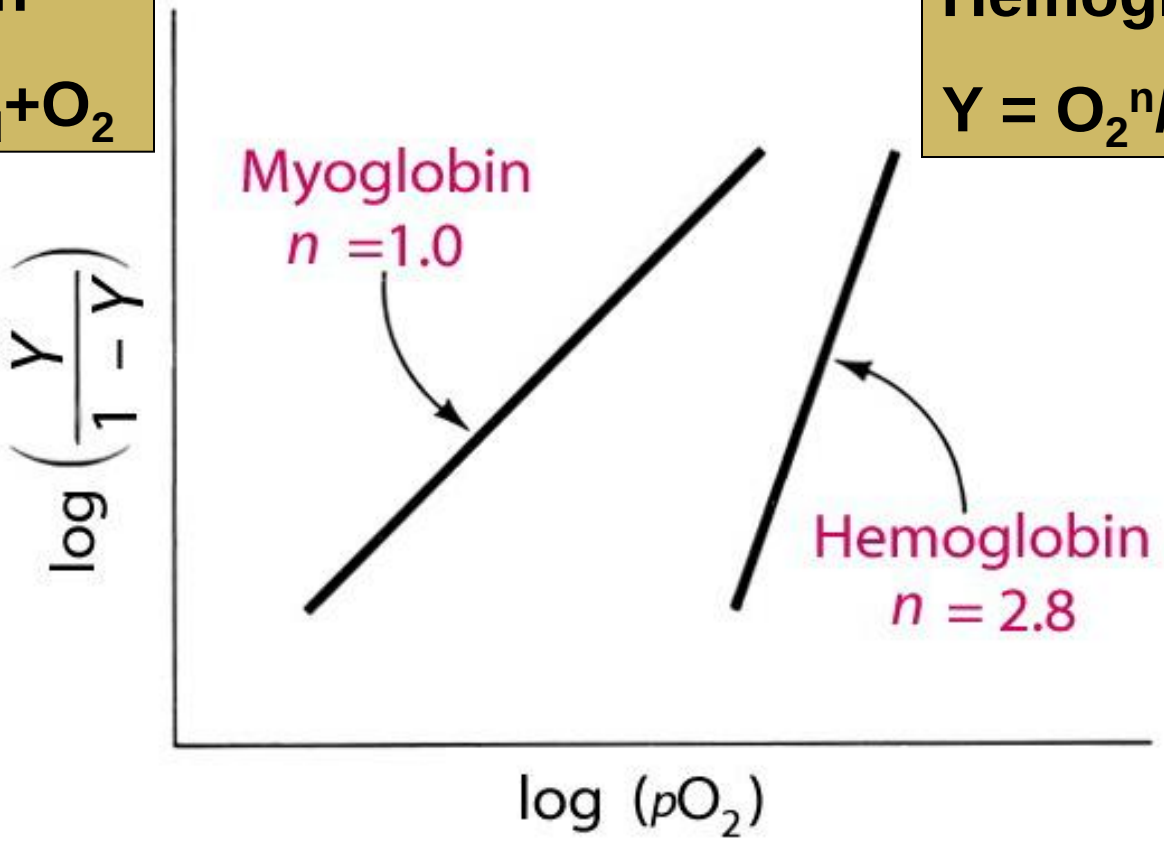
Cooperative Binding of Ligands

Myoglobin

$Y = O_2 / K_d + O_2$

Hemoglobin

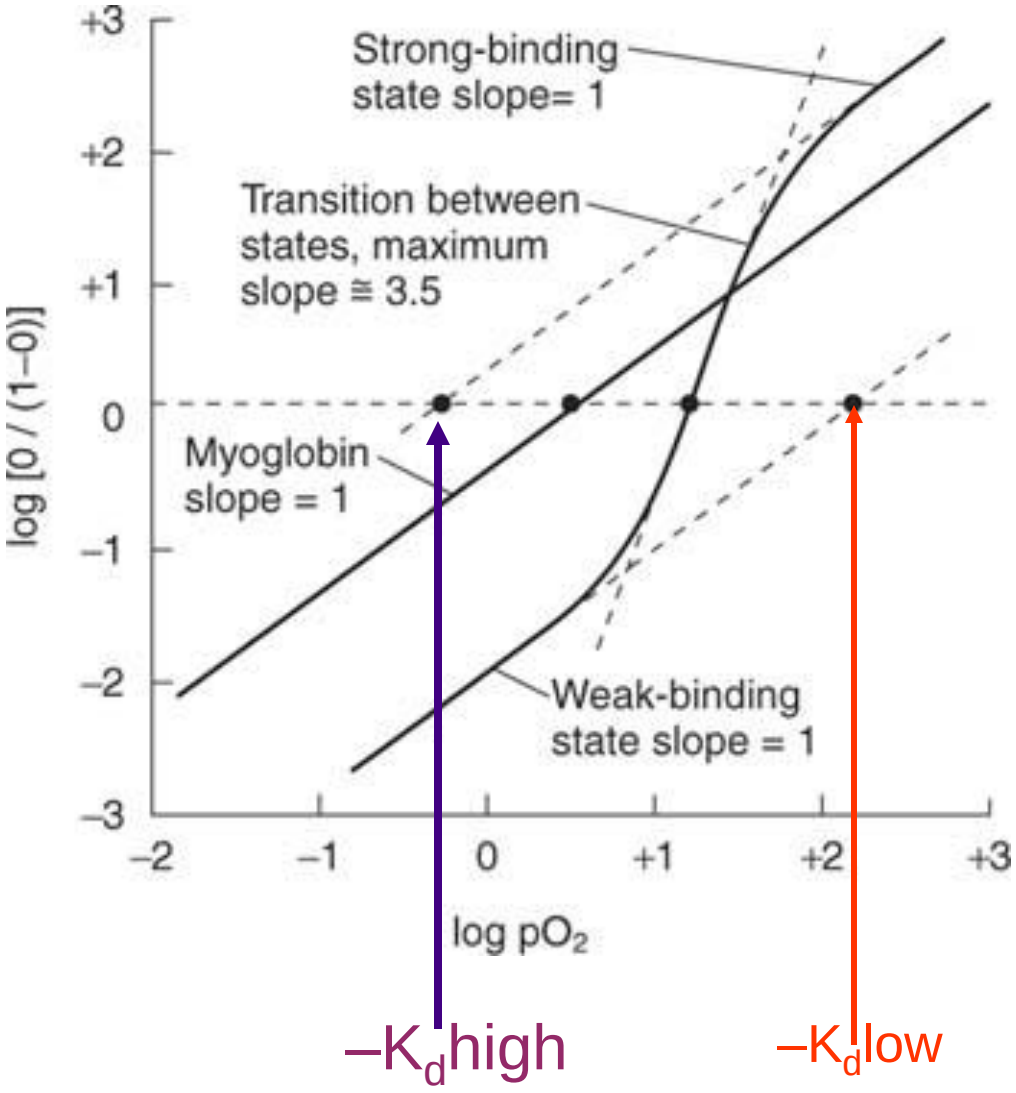
$Y = O_2^n / K_d + O_2^n$



Hill plots: Slope measures degree of cooperativity

$\text{Log } [Y/(1-Y)] = n \log (pO_2) - \log Kd$

Cooperative Binding of Ligands Hill Plots



- θ=0.5, 1- θ =0.5
  - log(0.5/0.5)=log(1)=0
  - Evaluate slope at x intercept to evaluate n_H
  - Y intercept = -log $K_{doverall}$

Extrapolation the edges of a Hill plot (where slope = 1) to y=0 to determine K_{dhigh} and K_{dlow}

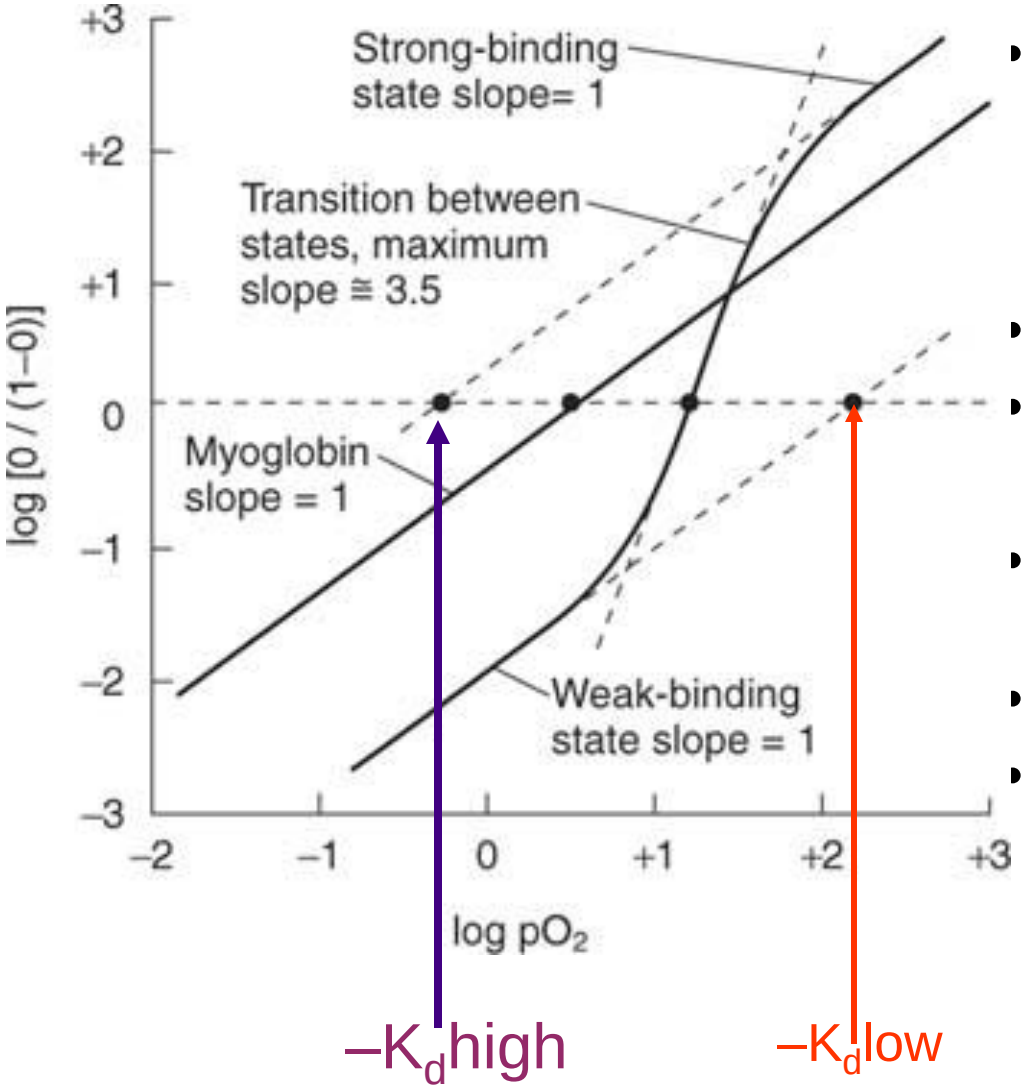
High saturation, Prod>Reac, all high affinity; slope =1

- extrapolate to Y=0
- $nlogO_2 = log\ O_2 = log\ K_{dhigh}$
- $K_{dhigh} = [O_2] = 0.50$ torr

Low saturation, Prod<React, all low affinity sites; slope = 1

- Extrapolate to Y=0
- $nlogO_2 = log\ O_2 = log\ K_{dlow}$
- $K_{dlow} = [O_2] = 105$ torr

Cooperative Hill Plot



- Calculate the binding energies for high and low affinity states of Hb
- $\Delta G_{high} = -RT\ln(1/K_{d\ high})$
- $\Delta G_{low} = -RT\ln(1/Kd_{\ low})$
- difference is the energy of cooperativity
- $\Delta\Delta G = -RT\ln(K_{dlow}/K_{dhigh})$
- $\Delta\Delta G = -RT\ln(105/0.500)$

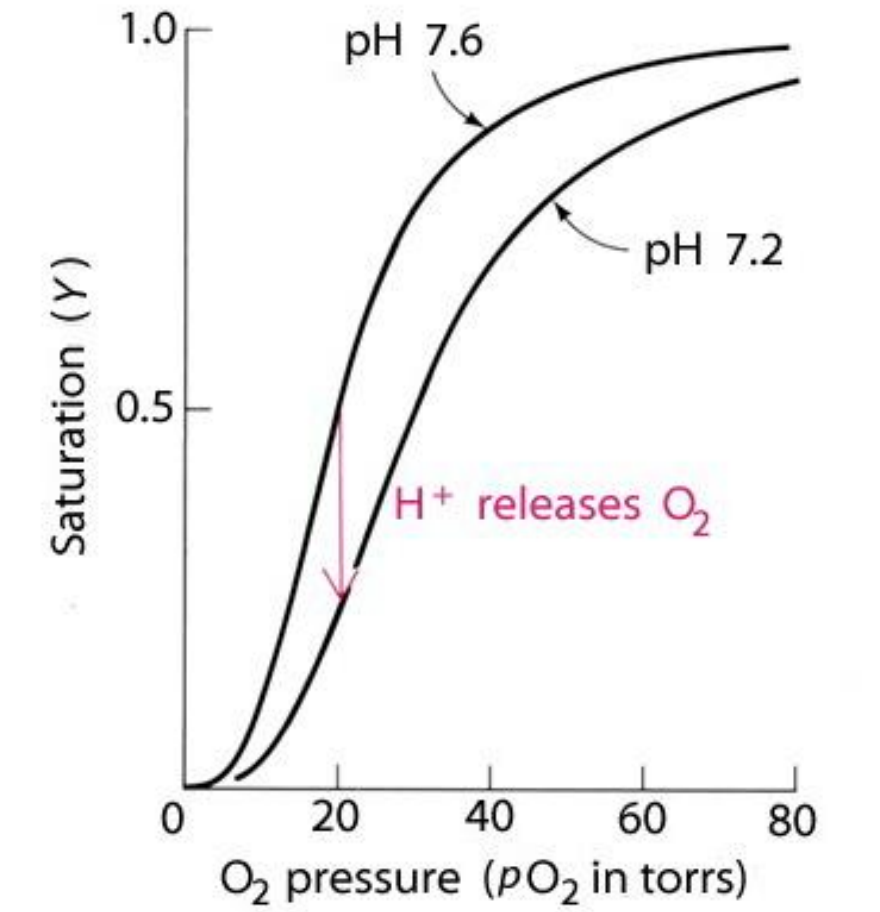
Molecular Associations: cooperativity/allostery

- allostery: indirect interaction that acts as a switch and modulates the affinity of a protein for its ligand, or increases the activity of an enzyme. If switch is ligand itself, cooperativity

- Example: one binding site, one conformational change under control of external switch from low affinity T state to high affinity R state

- $T_o + L \rightleftarrows T_1$ with affinity constant K_T
- $R_o + L \rightleftarrows R_1$ with affinity constant K_R
- $T_o \rightleftarrows R_o$ with conformational constant Y_o
- $T_1 \rightleftarrows R_1$ with conformational constant Y_1

Molecular Associations: allostery

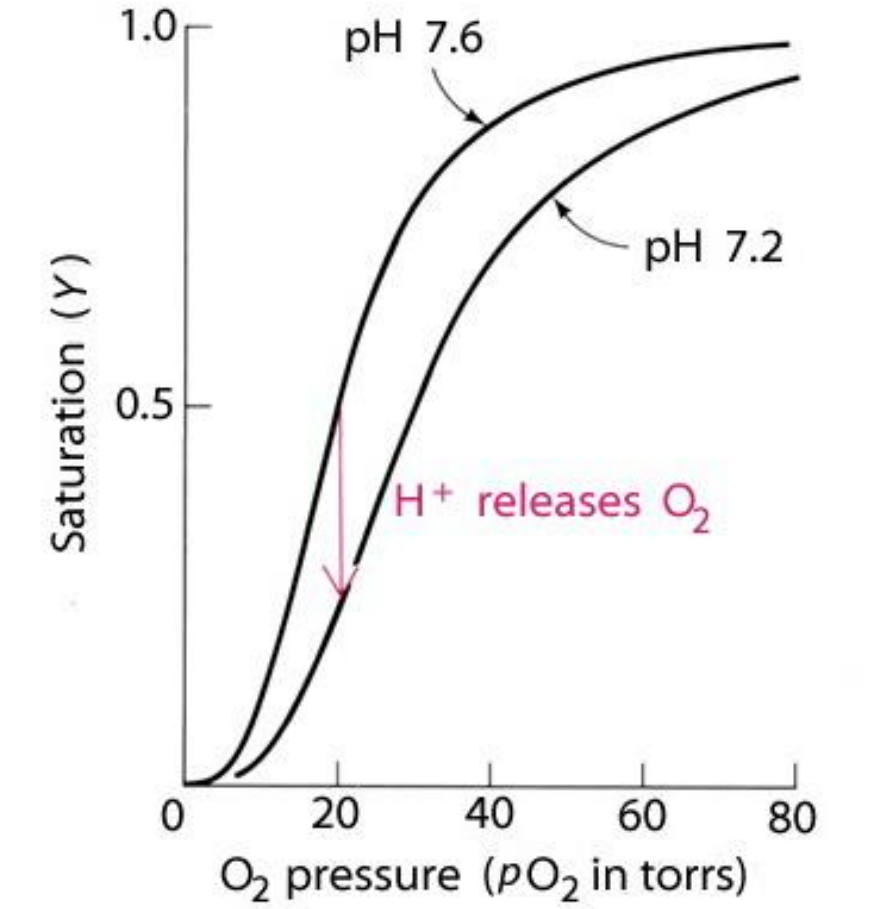


Pasteur Effect H+ is an allosteric effector

* **Allosteric Regulators** are molecules or atoms which affect the function of a protein from afar, that is they do not bind at the active site, but elsewhere on the protein.

* Protons are allosteric effectors and decrease affinity of Hb for oxygen, Bohr Effect.

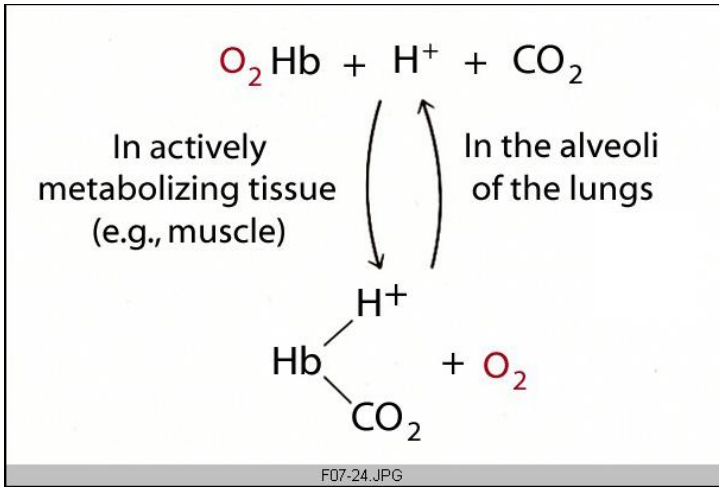
Molecular Associations: allostery



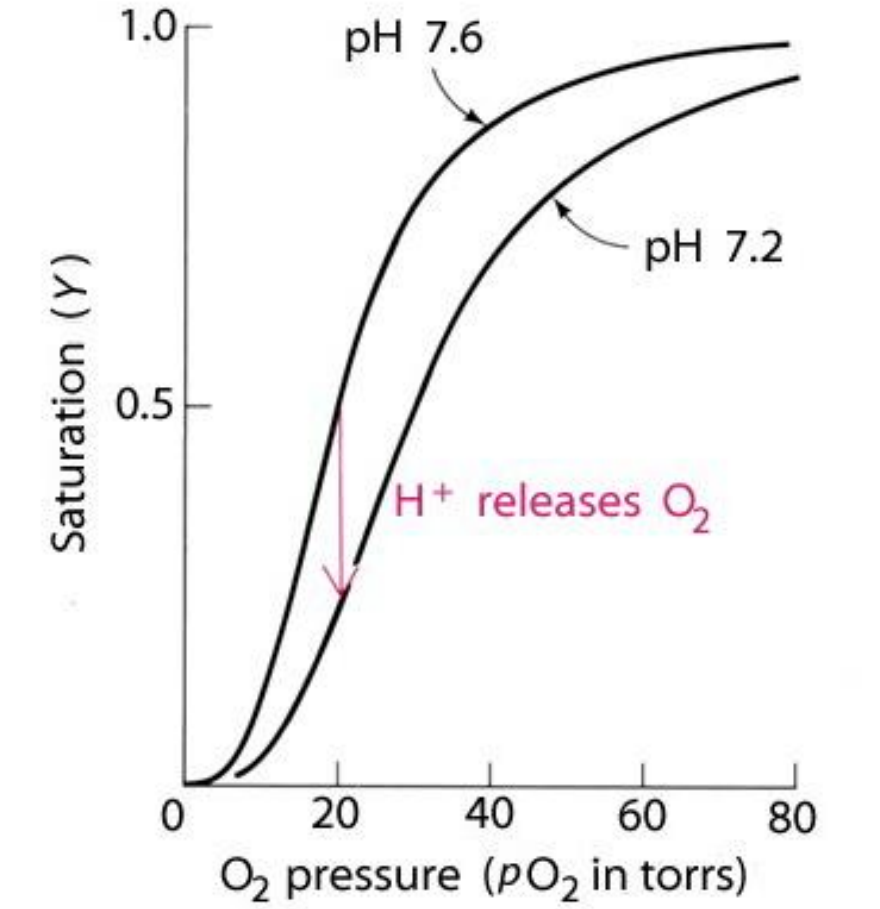
Pasteur Effect H+ is an allosteric effector

* Protons are allosteric effectors and decrease affinity of Hb for oxygen

* in deoxy, H^+ binds to His-β146, His-α122, α-amino group of alpha chains.



Molecular Associations: allostery



Pasteur Effect H+ is an allosteric effector

* Protons are allosteric effectors and decrease affinity of Hb for oxygen

* in deoxy, H^+ binds to His-β146, His-α122, α-amino group of alpha chains.