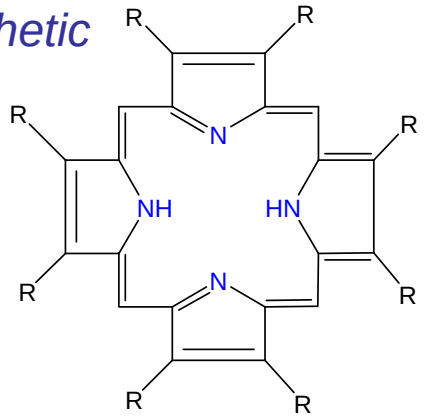
Protein conformation, dynamics and function

Key principles:

- Protein function involves binding of other molecules "Ligand"
- Proteins contain ligand "binding sites"
  - highly specific
  - single or many sites
  - 1 or more different ligand type
- Proteins are not rigid "conformational change"
- Conformational changes are often linked to ligand binding "induced fit"
- Conf. change, site 1 ←→ conf. change, site 2
- Protein-ligand interactions may be regulated

Oxygen binds to a heme prosthetic group

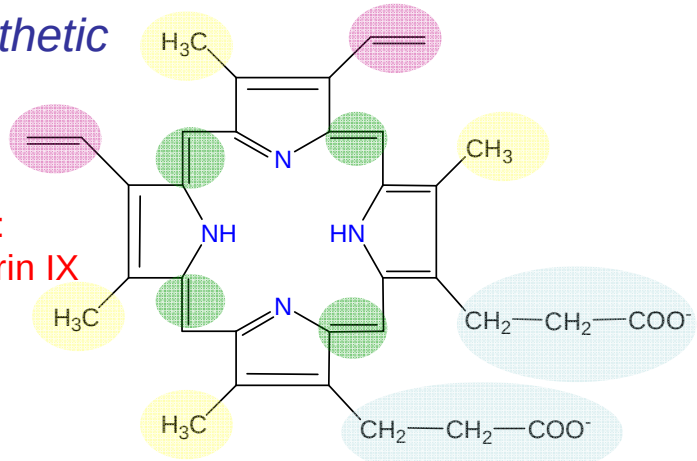
"tetrapyrrole ring"



Oxygen binds to a heme prosthetic group

Vinyl side chain

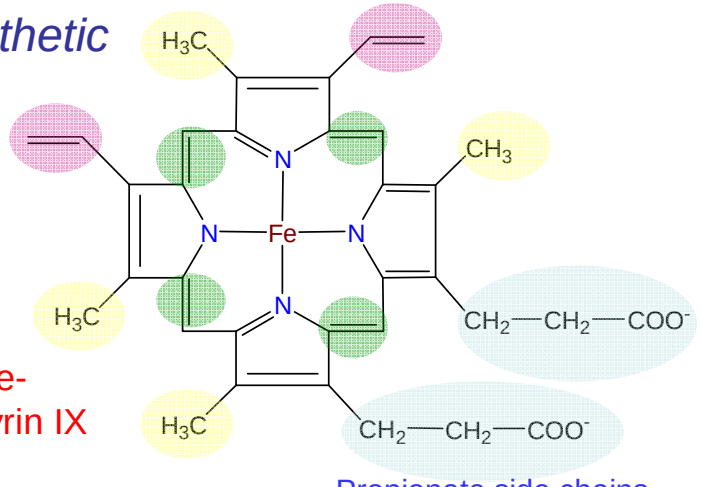
Without iron: protoporphyrin IX



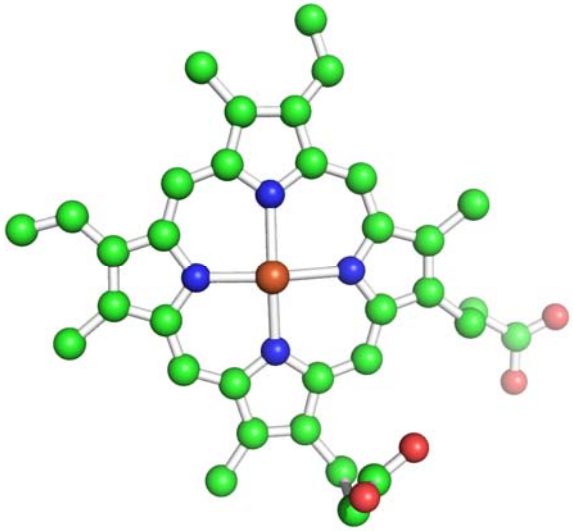
Methene bridges

Propionate side chains

Oxygen binds to a heme prosthetic group

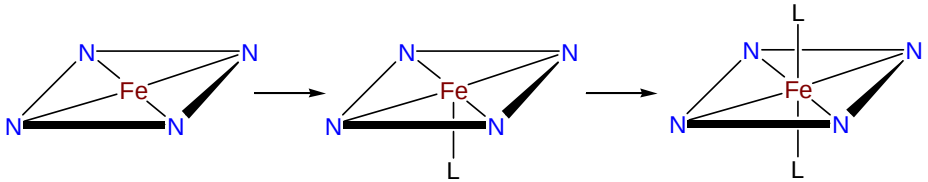


With iron: Fe-protoporphyrin IX (heme)

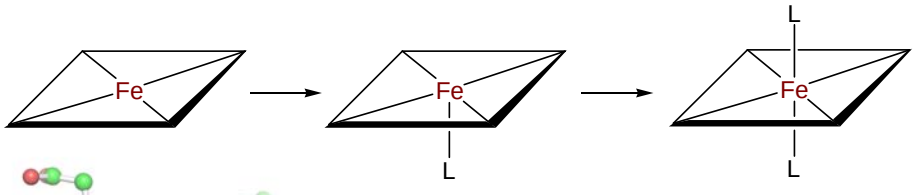


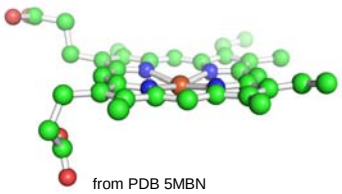
from PDB 5MBN

5th and 6th coordination sites on iron



square planar | square pyramidal | octahedral

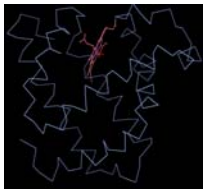




from PDB 5MBN

Myoglobin

- O_2 storage protein
- 3-D structure: globin fold, 75% α-helix
- Single polypeptide, Mr = 16,700, 153 AA
- 1 heme/polypeptide
- Interior is hydrophobic except for 2 his residues



Lopez.kin

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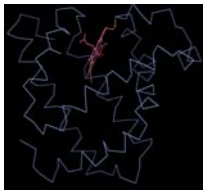
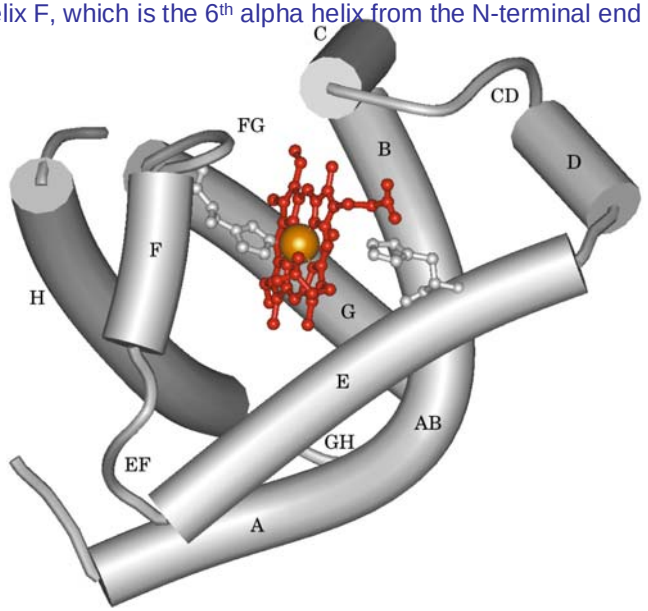
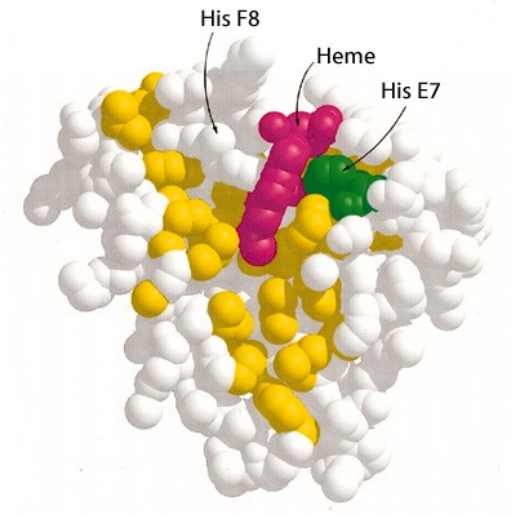


Lopez.kin

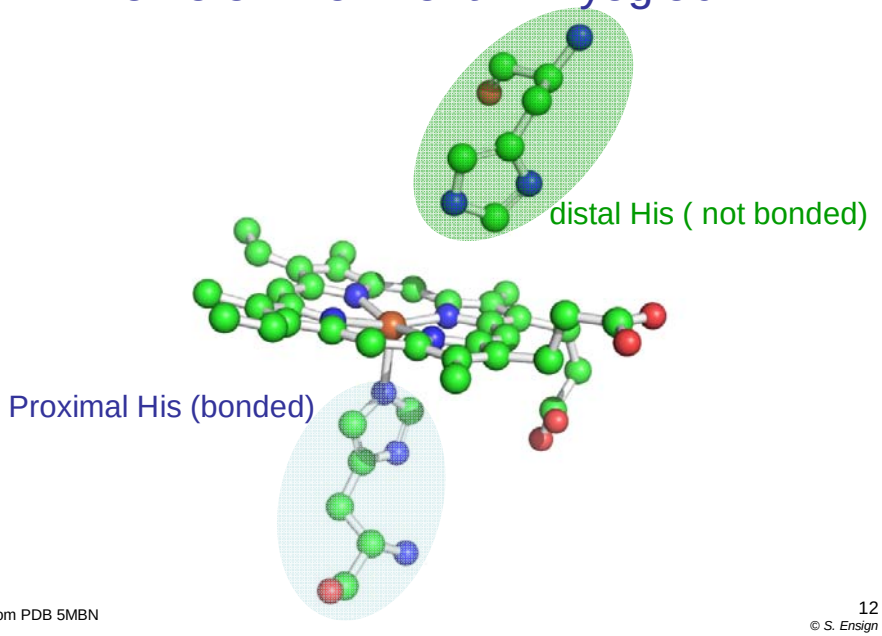
Note helix designations using letters. Residue "F8" is the eighth residue in helix F, which is the 6th alpha helix from the N-terminal end



Note helix designations using letters. Residue "F8" is the eighth residue in helix F, which is the 6th alpha helix from the N-terminal end



Heme environment in myoglobin



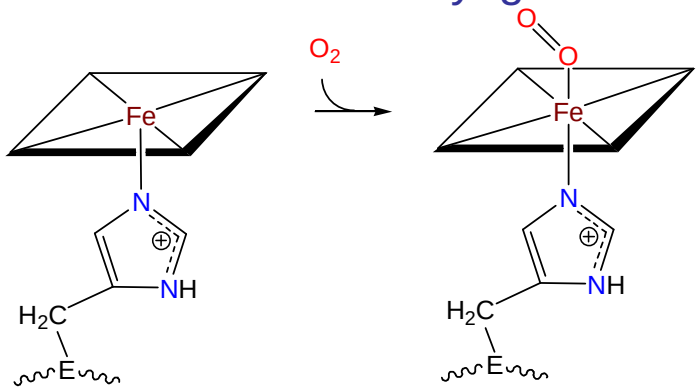
from PDB 5MBN

The occupation status of the 6th coordination site is critical to the physiological status of myoglobin and hemoglobin

Form	Oxidation state	Ligand at 6th position
Deoxy Mb	Fe^{2+}	None
Oxy Mb	Fe^{2+}	O_2
Ferri Mb	Fe^{3+}	H_2O

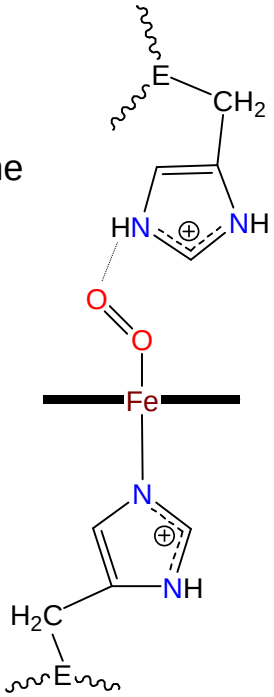
O_2 can only bind ferrous heme!

Binding of oxygen to the 6th coordination site of heme in myoglobin

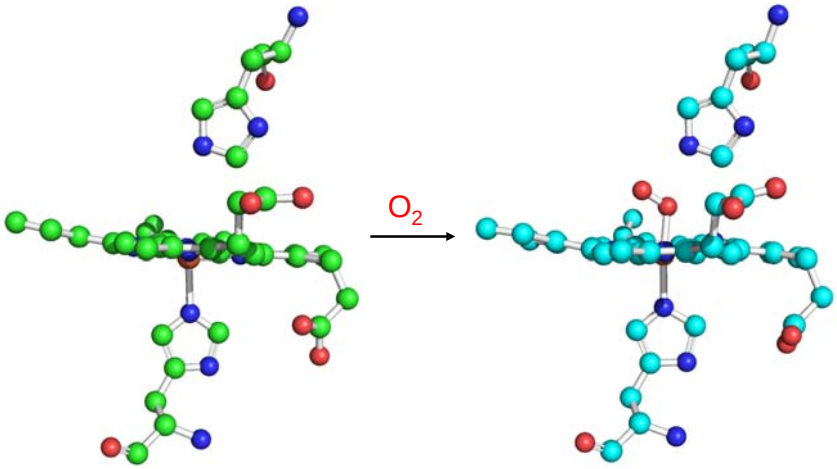


O_2 is sp^2 hybridized, and thus binds to Fe at a 120 degree angle

O_2 is sp^2 hybridized, and thus binds to Fe at a 120 degree angle, which is facilitated by the position of the distal histidine



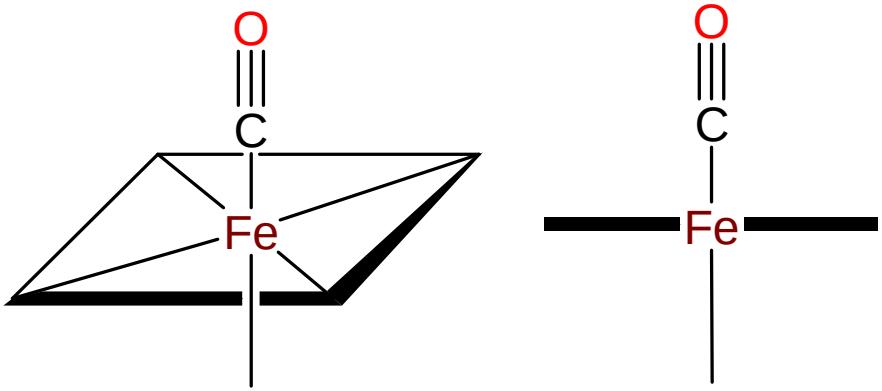
Binding of oxygen to the 6th coordination site of heme in myoglobin



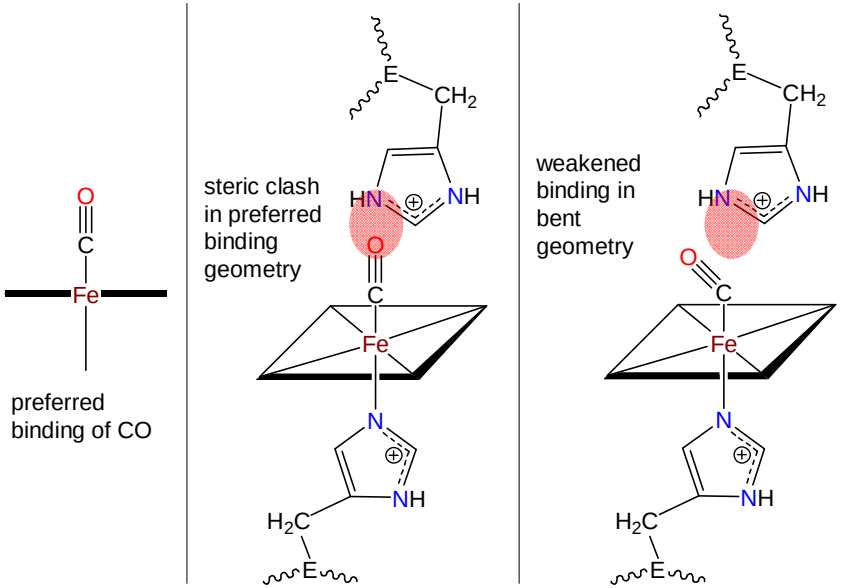
from PDB 5MBN

from PDB 1MBO

CO has a high affinity for the iron in heme



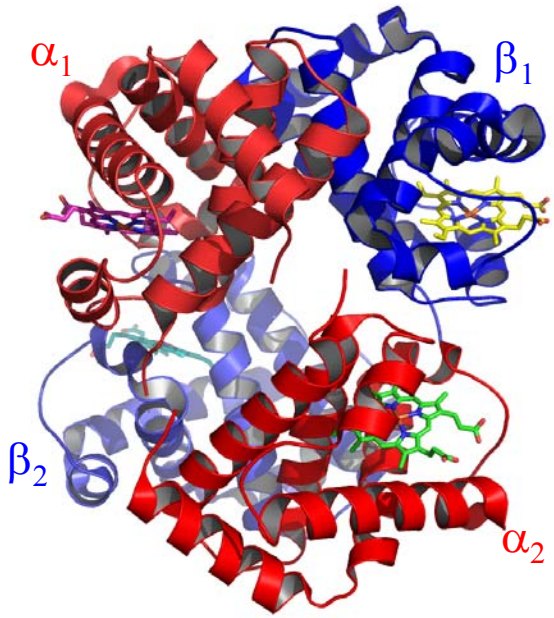
The distal His prevents linear binding of CO, which is preferred for the sp hybridized CO molecule



Hemoglobin:

$\alpha_2\beta_2$ tetramer

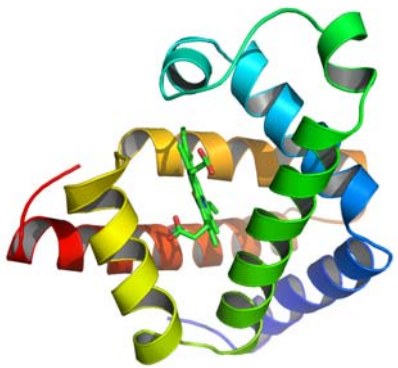
O_2 transport



from PDB 2hhb

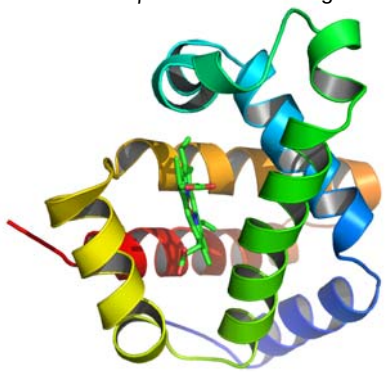
Myoglobin and the subunits of hemoglobin are built from the same structural motifs

myoglobin



from PDB 5MBN

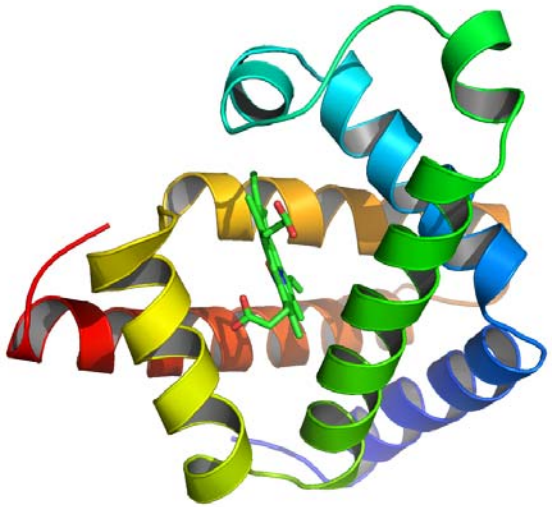
β subunit of hemoglobin



from PDB 2hhb

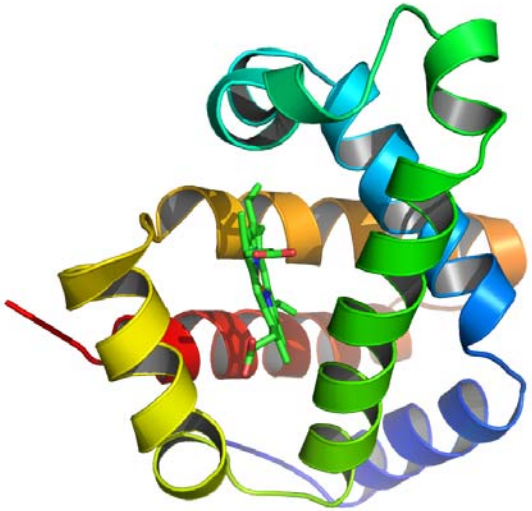
AA sequences are only “identical” at 24 of 141 positions!

“Fold” structure of myoglobin



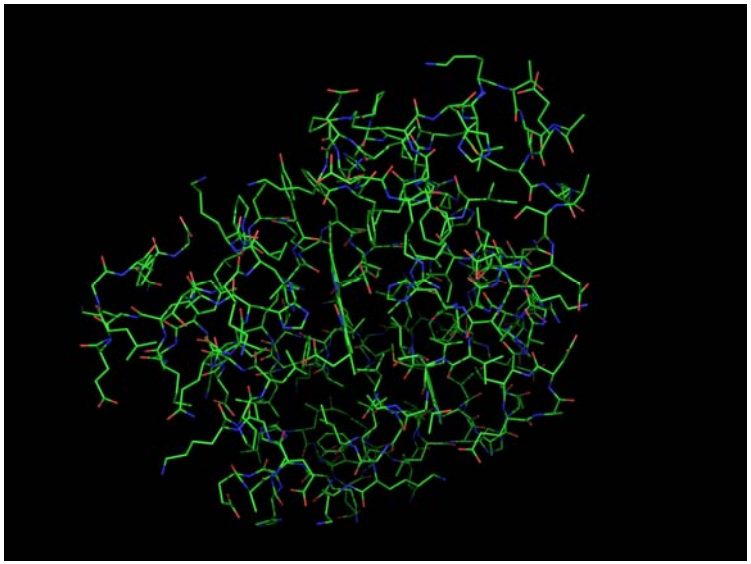
from PDB 5MBN

“Fold” structure of beta subunit of hemoglobin



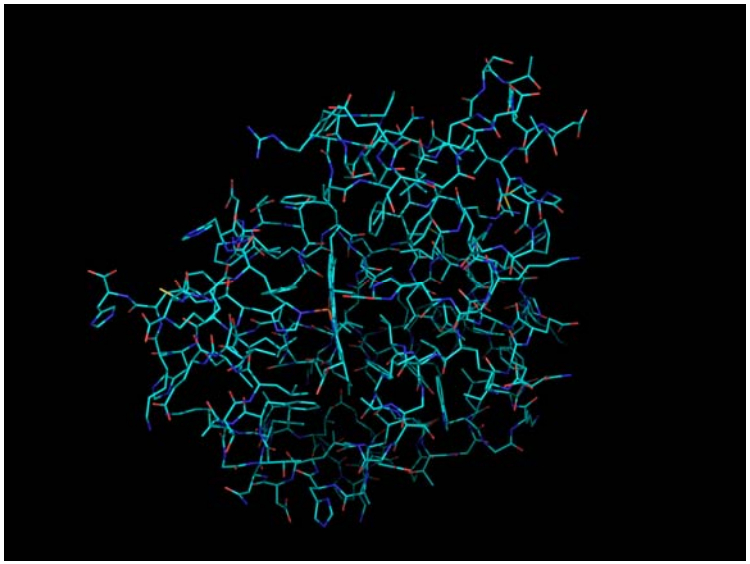
from PDB 5hhb

Complete structure of myoglobin



from PDB 5MBN

Complete structure of beta subunit of hemoglobin



from PDB2hhb

Different amino acid sequences can specify similar 3-D structures

- Some residue positions are “highly conserved” (identical)
  - His93, His64, 7 others
- Some residue positions allow conservative changes (similar AA)
  - Nonpolar residues in interior (ala → ile)
- Some residue positions are not conserved (high variability)
  - Hydrophilic surface residues

Allosteric interactions: interactions between spatially distinct sites

- Hb is an allosteric protein, Mb is not
- Binding of O_2 to one site on Hb enhances binding of additional O_2 to other sites on the same molecule “cooperativity”
- Other molecules affect the affinity of O_2 for Hb:
  - pH
  - CO_2
  - 2,3-diphosphoglycerate

Mathematical and graphical representations of ligand binding to proteins

A hyperbola describes the simple noncooperative binding of a ligand to a protein

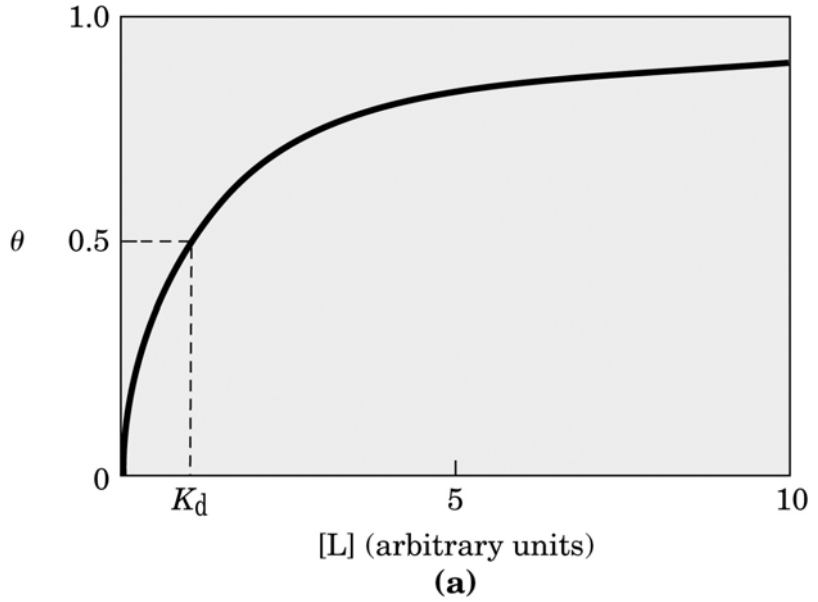


table 7–1 The lower the K_D, the tighter the binding

Some Protein Dissociation Constants

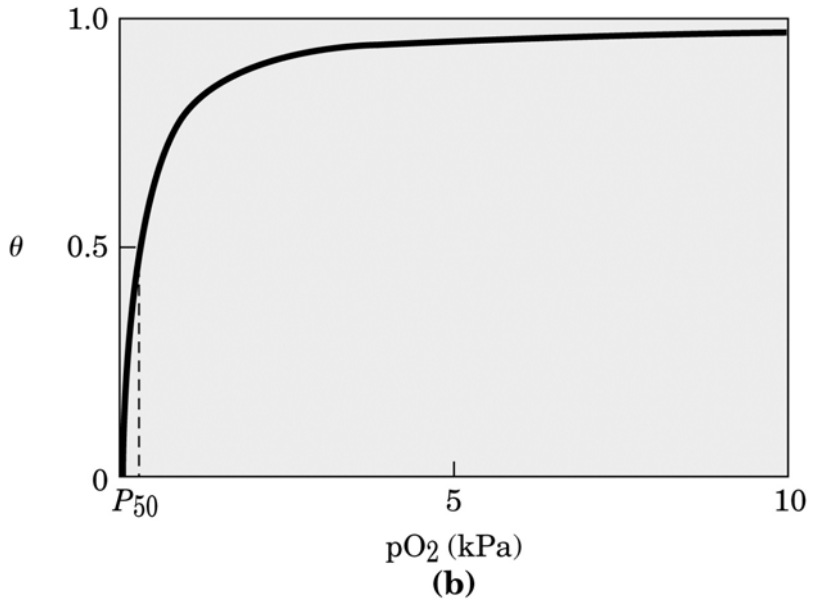
Protein	Ligand	K_d (M)*
Avidin (egg white)†	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)‡	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)§	Ca^{2+}	3×10^{-6}
		2×10^{-5}

*A reported dissociation constant is valid only for the particular solution conditions under which it was measured. K_d values for a protein-ligand interaction can be altered, sometimes by several orders of magnitude, by changes in solution salt concentration, pH, or other variables.

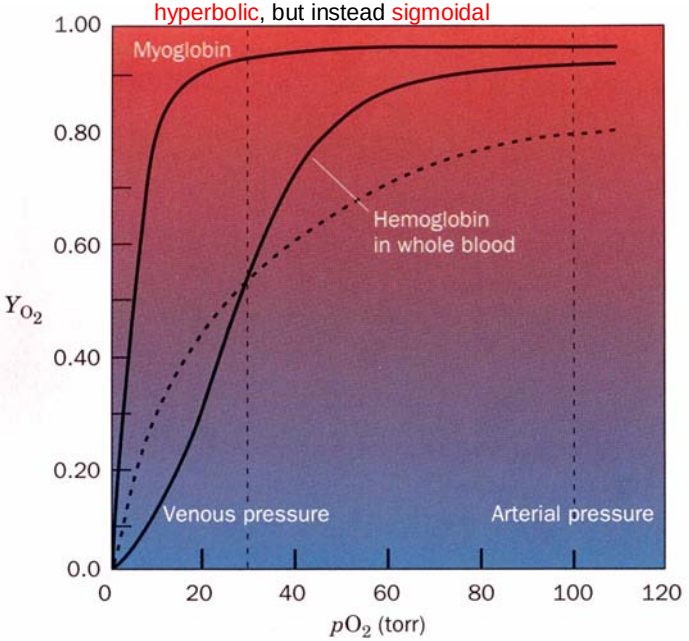
†Interaction of avidin with the enzymatic cofactor biotin is among the strongest noncovalent biochemical interactions known.

‡This immunoglobulin was isolated as part of an effort to develop a vaccine against HIV. Immunoglobulins (described later in the chapter) are highly variable, and the K_d reported here should not be considered characteristic of all immunoglobulins.

§Calmodulin has four binding sites for calcium. The values shown reflect the highest- and lowest-affinity binding sites observed in one set of measurements.

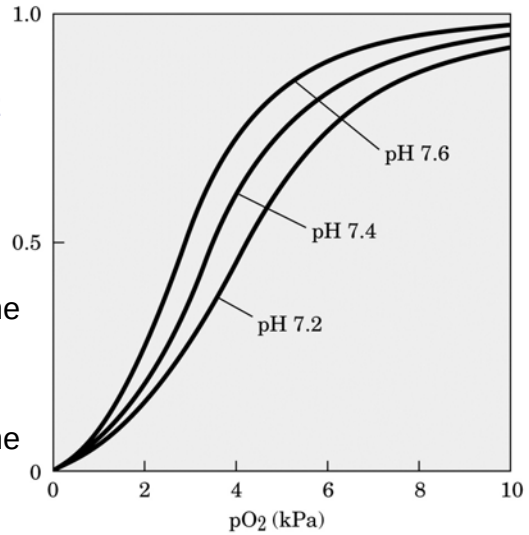


The oxygen dissociation curve for hemoglobin is not hyperbolic, but instead sigmoidal



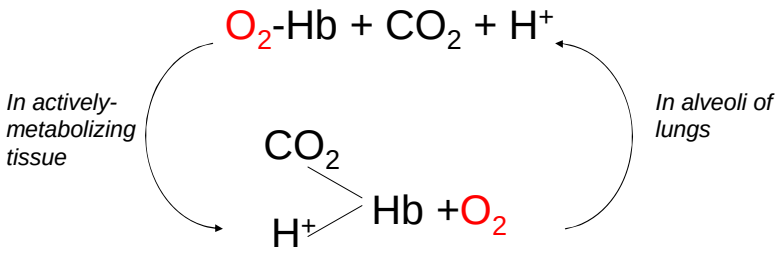
The Bohr effect: Effects of CO_2 and $[H^+]$ on O_2 release from hemoglobin

- Increased $[H^+]$ shifts dissociation curve to the right
- Increased $[CO_2]$ shifts dissociation curve to the right

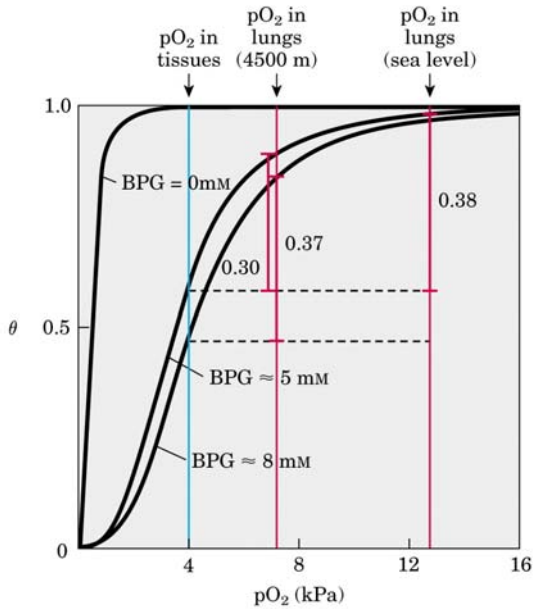


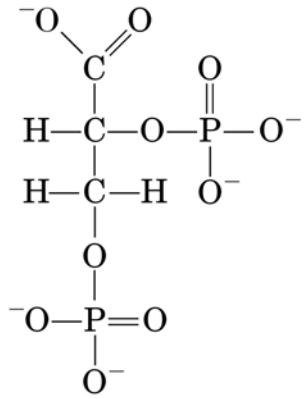
Increased CO_2 and H^+ concentrations lower O_2 affinity for Hb, i.e. promoting release of O_2 from oxyhemoglobin

Hemoglobin transports CO_2 and H^+ as well as O_2



2,3-DPG lowers the oxygen affinity of Hb

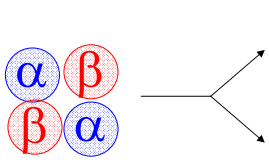


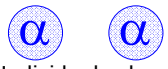


2,3-Bisphosphoglycerate

What is the biochemical basis for allosteric effects in hemoglobin?

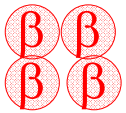
Expt. 1: Dissociate Hb into individual subunits





Individual subunits

No allostery by themselves



No allostery in β_4 tetramer

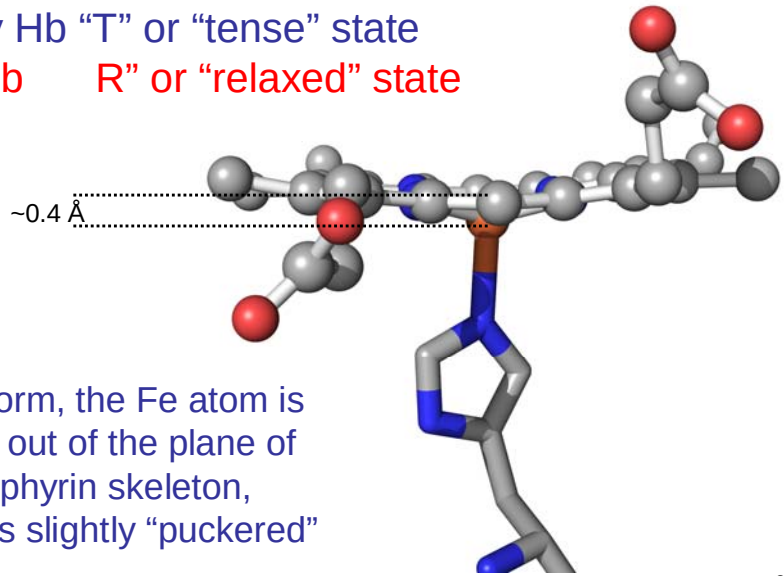
Associate together to make a β_4 teramer

Conclusion: The allosteric properties of Hb must arise from interactions between subunits in the native quaternary structure

Two conformations of Hb

Deoxy Hb "T" or "tense" state

Oxy Hb R" or "relaxed" state



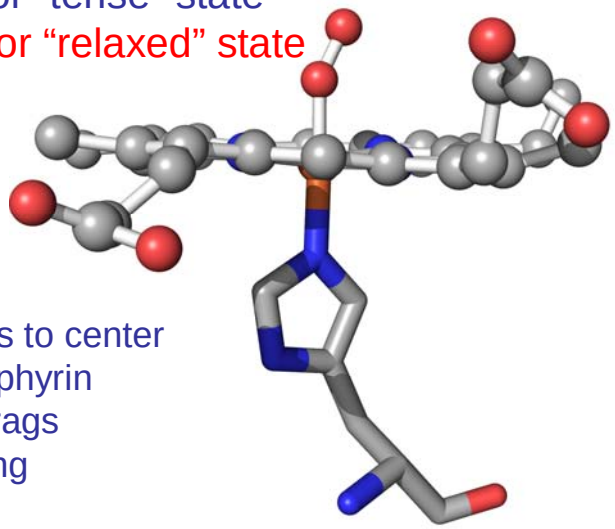
In "T" form, the Fe atom is slightly out of the plane of the porphyrin skeleton, which is slightly "puckered"

from PDB2hhb

Two conformations of Hb

Deoxy Hb "T" or "tense" state

Oxy Hb R" or "relaxed" state



T → R: Fe moves to center of porphyrin, porphyrin straigtens out, drags proximal His along

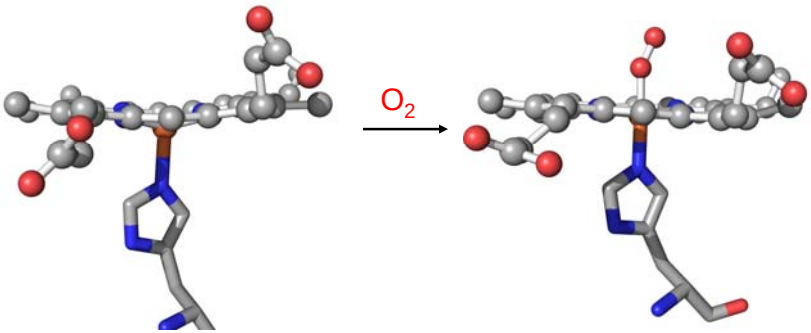
from PDB1hho

Two conformations of Hb

- Deoxy Hb "T" or "tense" state

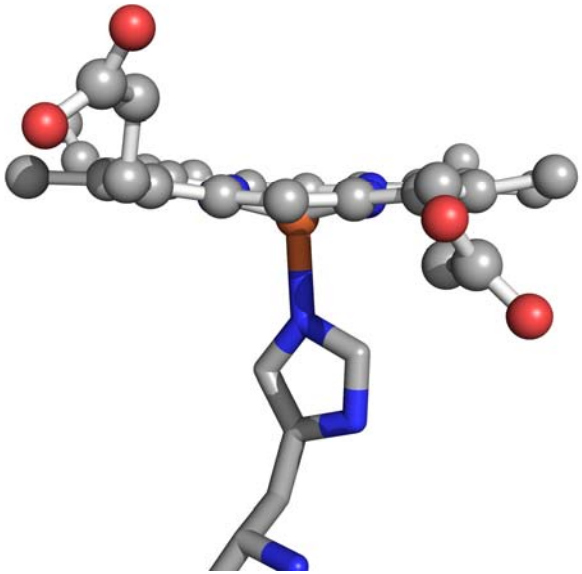
Oxy Hb "R" or "relaxed" state

T → R: Fe moves to center of porphyrin, drags proximal His along, this tilts attached F helix

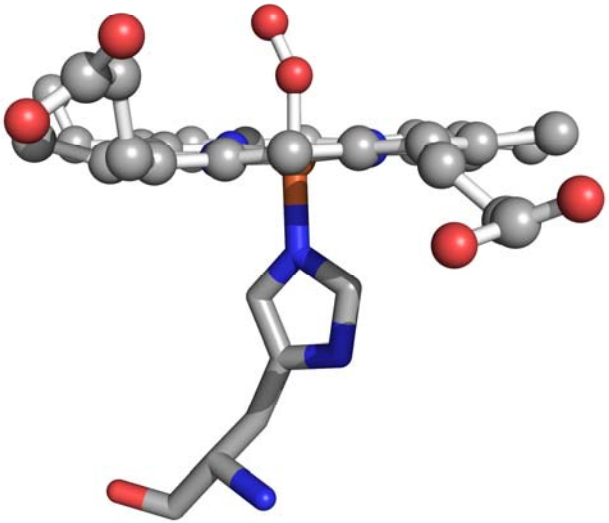


from PDB2hhb

from PDB1hho

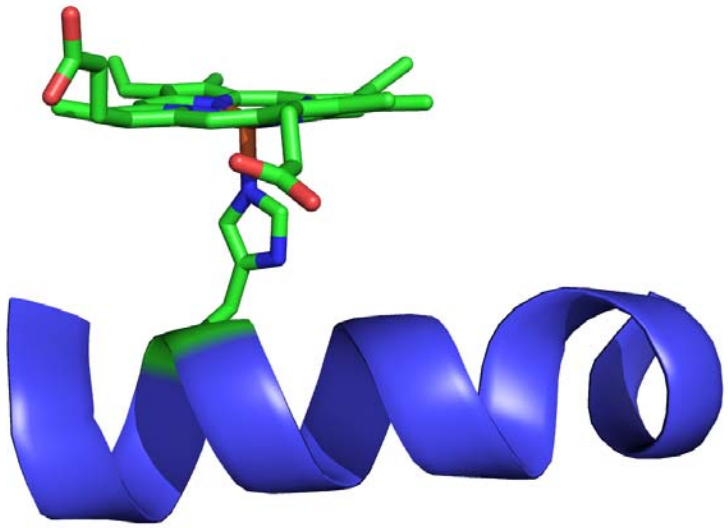


from PDB2hhb



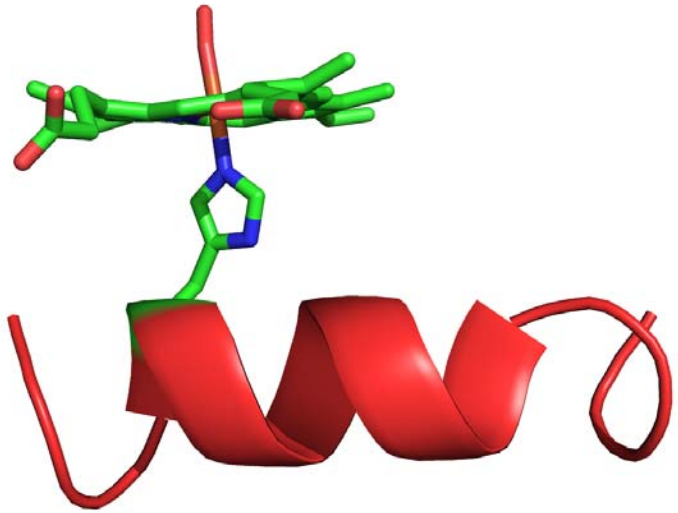
from PDB1hho

Note orientation of F helix with respect to heme in deoxyHb

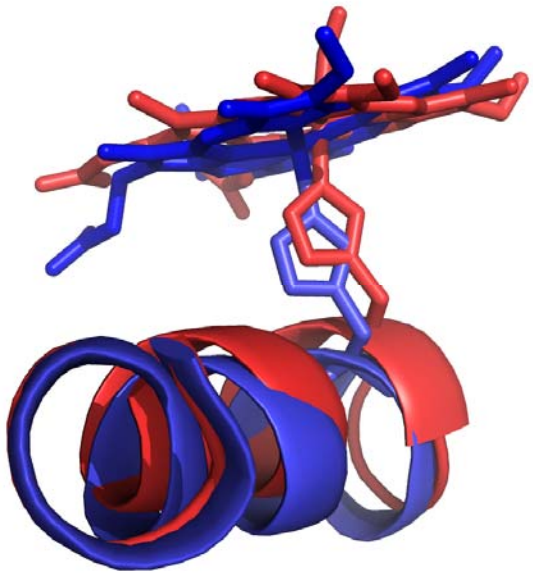


from PDB2hhb

Note orientation of F helix with respect to heme in oxyHb

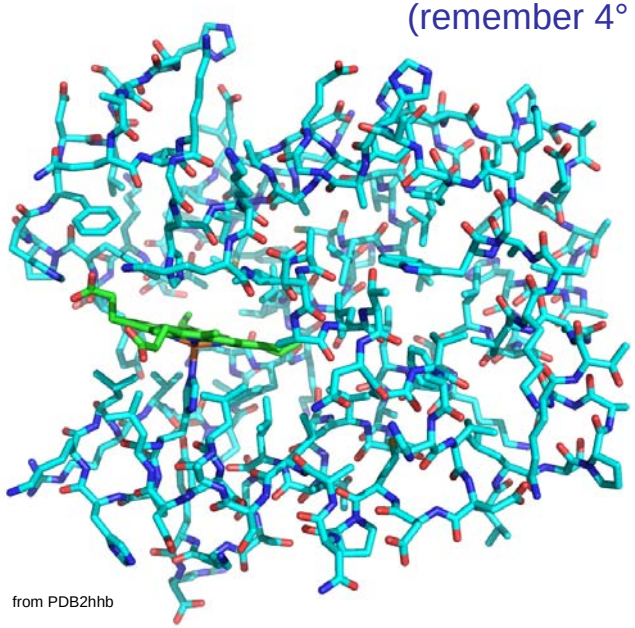


from PDB1hho



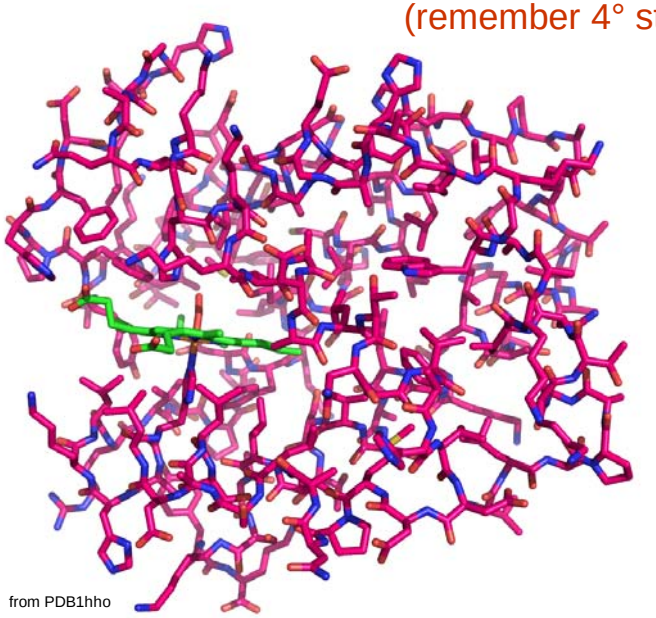
from PDB2hhb and 1hho

Conformation of a single alpha subunit in deoxyHb (remember 4° structure is $\alpha_2\beta_2$)



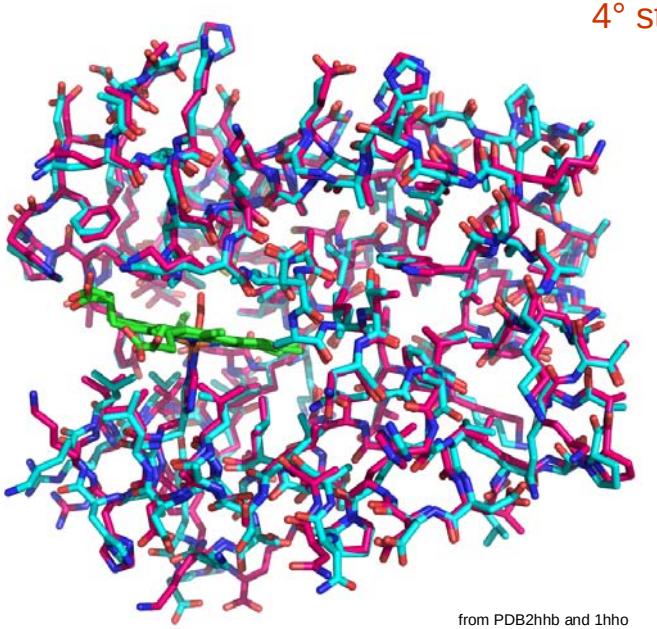
from PDB2hhb

Conformation of a single alpha subunit in oxyHb (remember 4° structure is $\alpha_2\beta_2$)



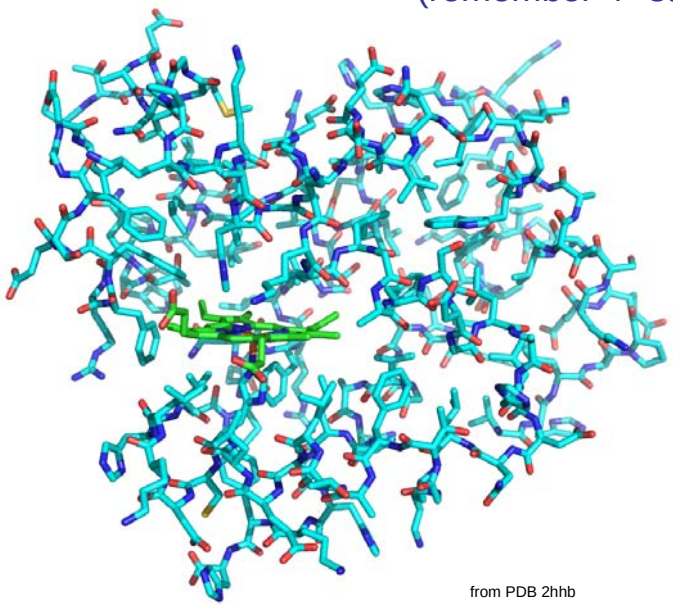
from PDB1hho

Overlay of deoxy and oxyHb alpha subunits (remember 4° structure is $\alpha_2\beta_2$)



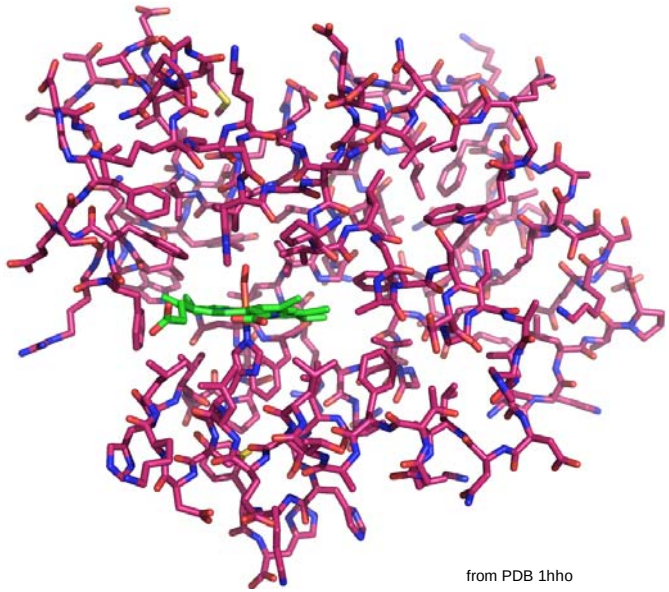
from PDB2hhb and 1hho

Conformation of a single beta subunit in deoxyHb (remember 4° structure is $\alpha_2\beta_2$)



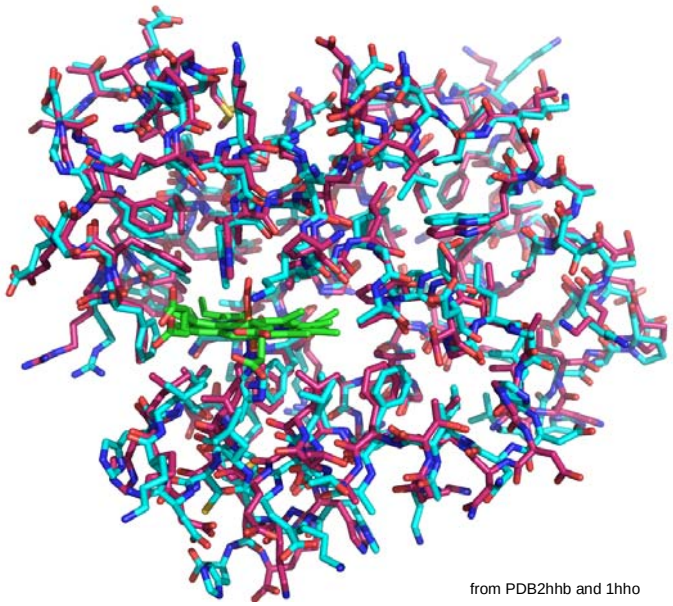
from PDB 2hhb

Conformation of a single beta subunit in oxyHb (remember 4° structure is $\alpha_2\beta_2$)



from PDB 1hho

Overlay of deoxy and oxyHb beta subunits (remember 4° structure is $\alpha_2\beta_2$)



from PDB2hhb and 1hho

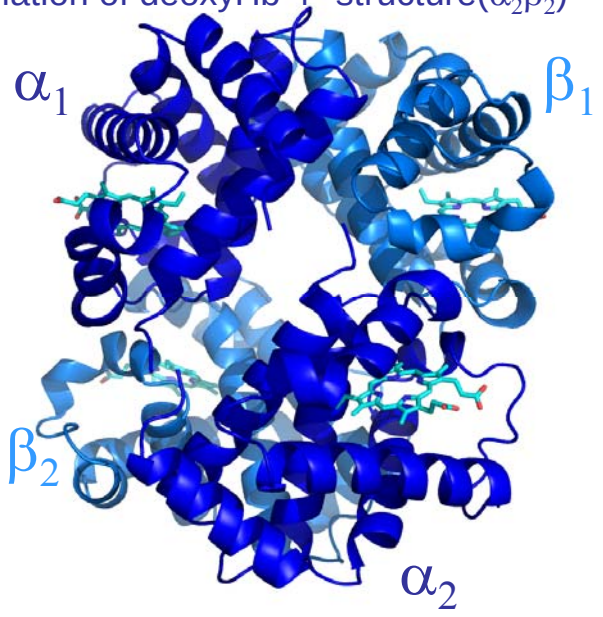
Summary of T and R hemoglobin structures

- T Hb (4° structure) is stabilized by multiple ionic interactions, H-bonds, and ion-dipole interactions within subunits (intrasubunit) or between subunits (intersubunit)
- O_2 binding to a "T" Hb subunit induces a conformational change in the heme, reorienting the F helix, which translates to an overall conformational change in the Hb subunit
- The change in conformation of the O_2-bound subunit leads to a reorientation/change of stabilizing interactions to adjacent subunits

Forces that stabilize T and R hemoglobin

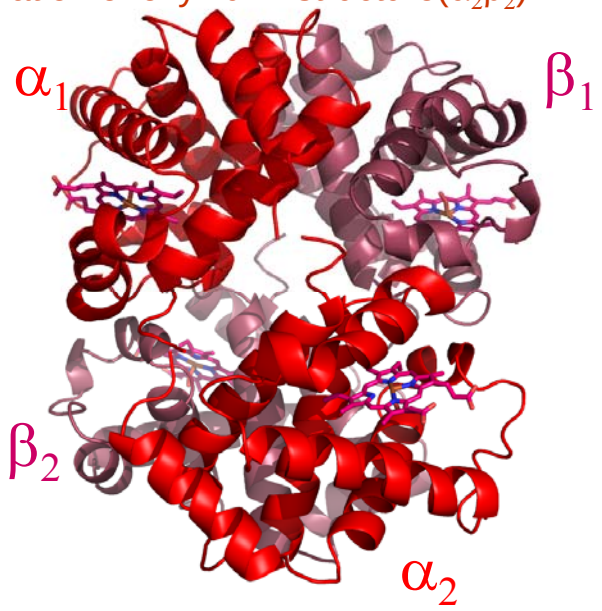
- Intra and inter subunit interactions
- interactions between amino acids
  - ionic interactions: "salt bridges"
    - ionic attractions between carboxy and amino termini and charged side chains
  - ion-dipole attractions
  - H bonds
- Salt bridges between amino acids and modified (carbamylated) amino acids (T only)
- Salt bridges between amino acid residues and 2,3-diphosphoglycerate (T only)

Conformation of deoxyHb 4° structure($\alpha_2\beta_2$)



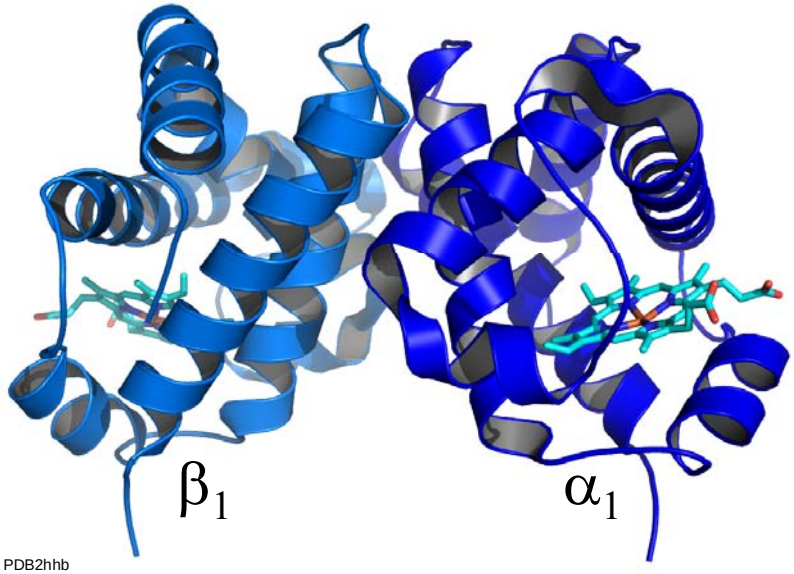
from PDB2hhb

Conformation of oxyHb 4° structure($\alpha_2\beta_2$)



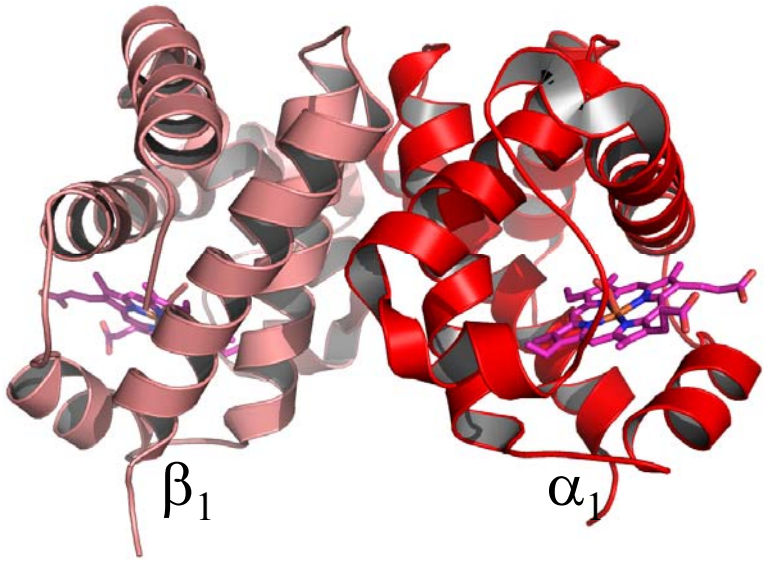
from PDB1hho

Conformation of deoxyHb ($\alpha_1\beta_1$)



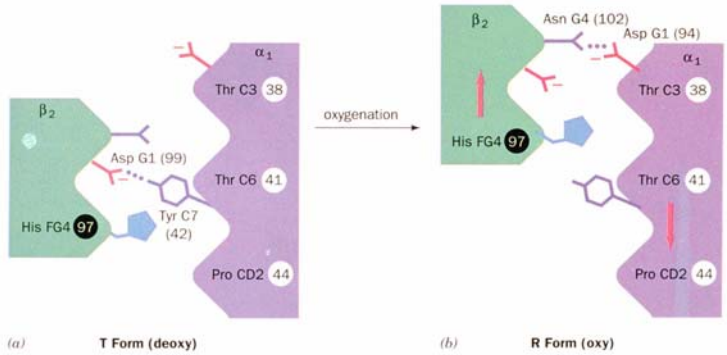
from PDB2hhb

Conformation of oxyHb ($\alpha_1\beta_1$)

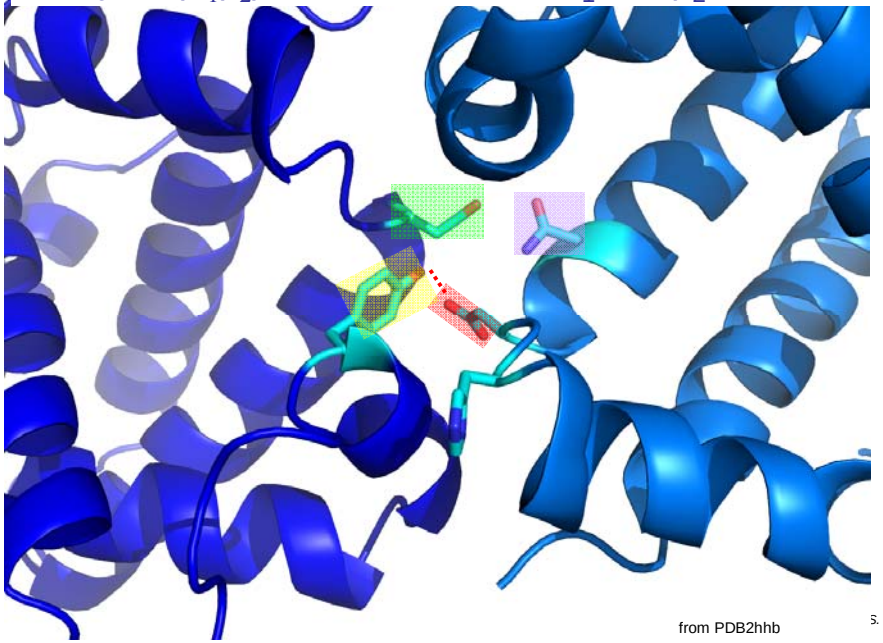


$\alpha 1-\beta 2$ contacts in the T and R states. Identical contacts are present at the $\alpha 2-\beta 1$ interface

Two different sets of H-bonds in 2 states: a switch, allowing only two stable positions of the subunits relative to each other

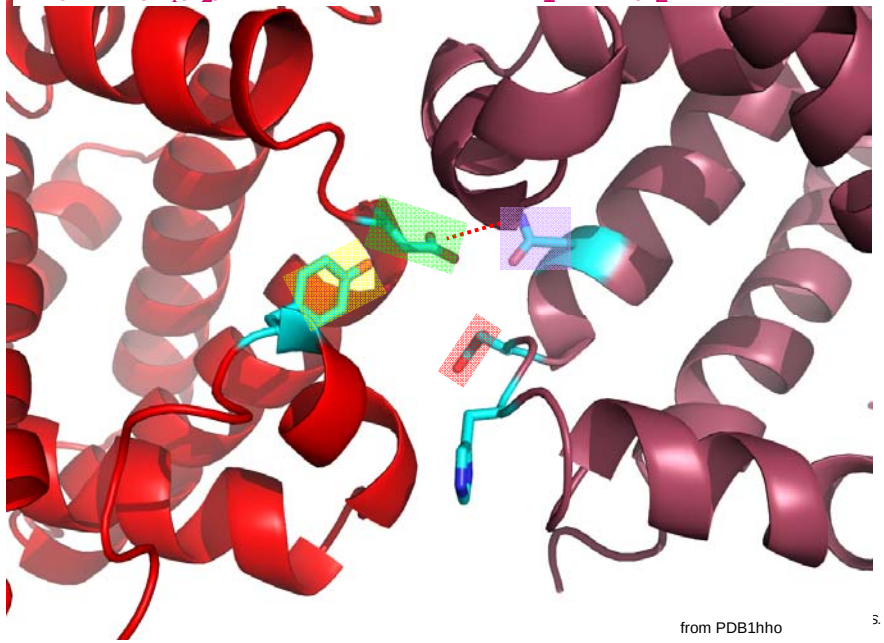


deoxyHb ($\alpha_1\beta_2$) subunit interface: α_1Y42-β_2D99 H-bond



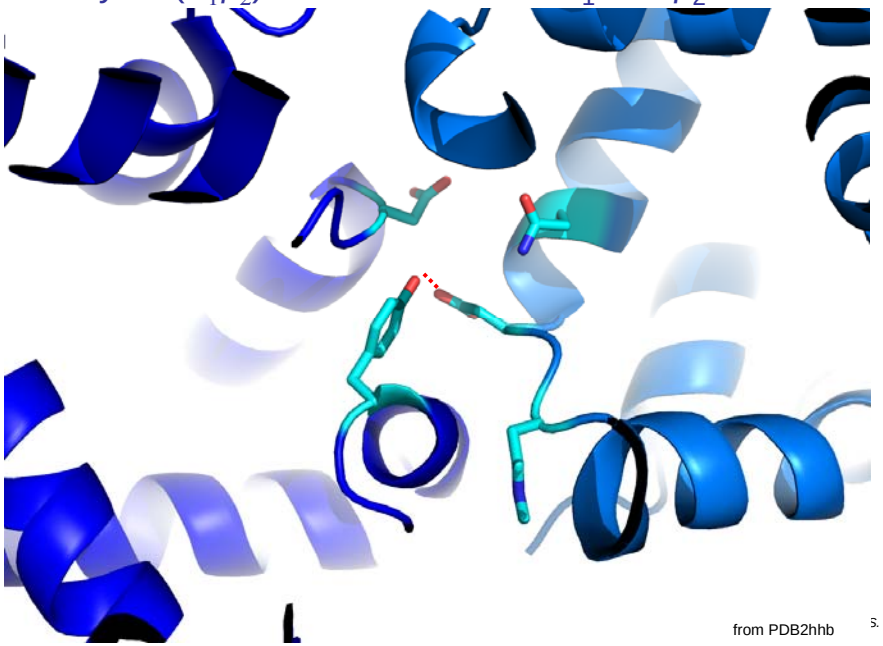
from PDB2hhb

oxyHb ($\alpha_1\beta_2$) subunit interface: α_1D94-β_2N102 H-bond



from PDB1hho

deoxyHb ($\alpha_1\beta_2$) subunit interface: α_1Y42-β_2D99 H-bond

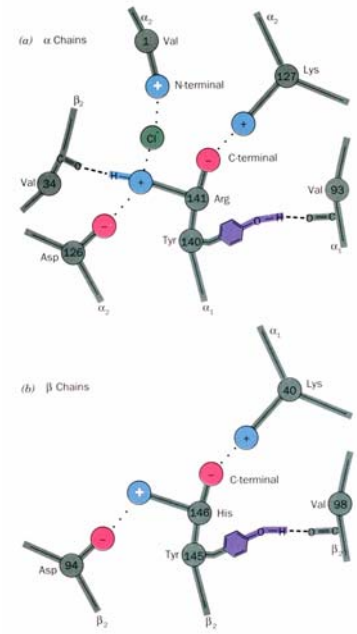


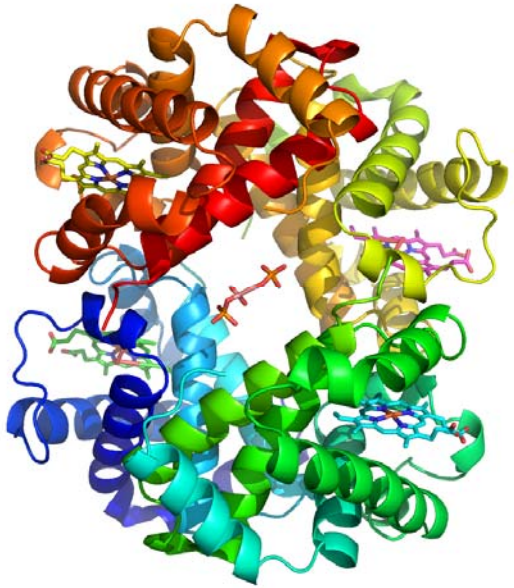
from PDB2hhb

oxyHb ($\alpha_1\beta_2$) subunit interface: α_1D94-β_2N102 H-bond

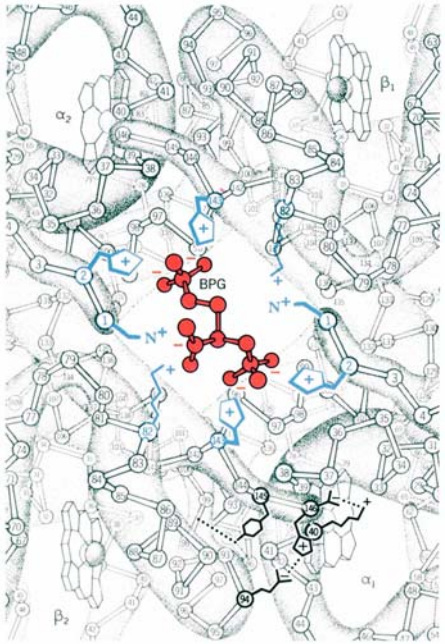
from PDB1hho

Some representative salt bridges in T Hb

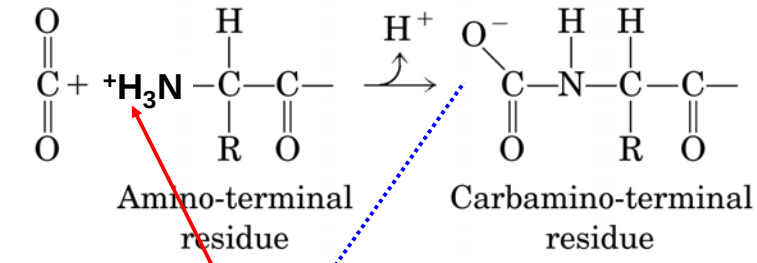




from PDB 1b86



Molecular explanation for the Bohr effect: CO_2



$AA—NH_3+$

A new salt brige: an electrostatic repulsion is converted to an electrostatic attraction!

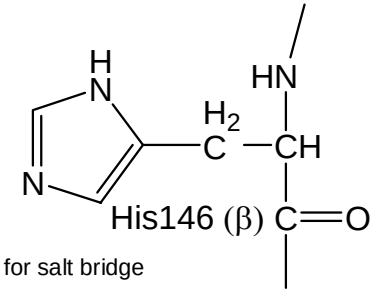
Molecular explanation for the Bohr effect: H^+

R (oxy) hemoglobin in lungs

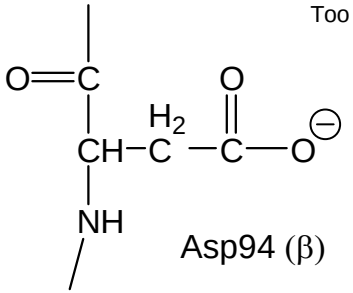
pH in lungs is ~7.4

His146 is deprotonated

(unperturbed His pKa = 6)



Too far apart for salt bridge



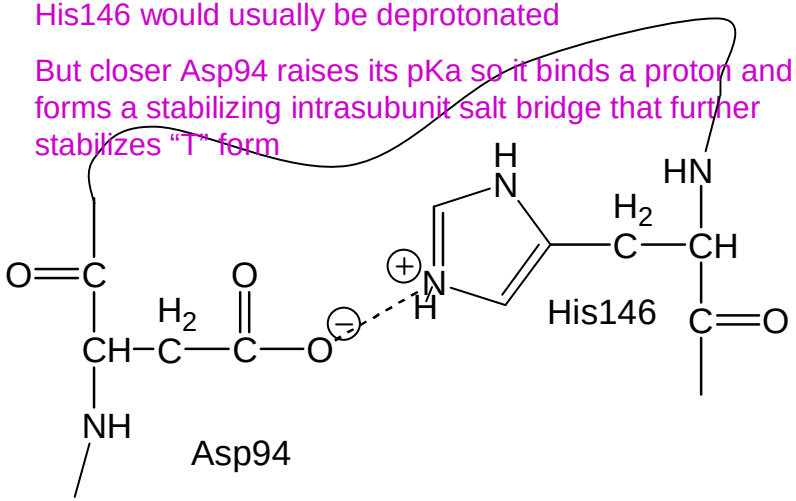
Molecular explanation for the Bohr effect: H^+

T hemoglobin in tissue

pH in tissue is ~7.2

His146 would usually be deprotonated

But closer Asp94 raises its pKa so it binds a proton and forms a stabilizing intrasubunit salt bridge that further stabilizes "T" form

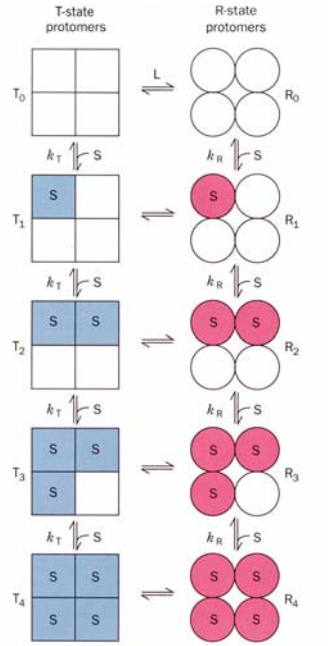


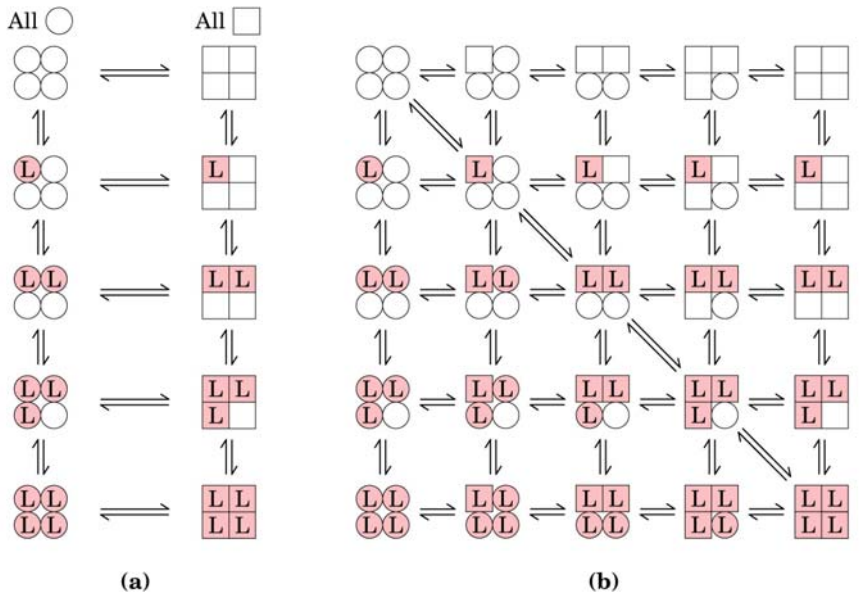
Summary of T and R hemoglobin structures

- T Hb (4° structure) is stabilized by multiple salt-bridges, H-bonds, and ion-dipole interactions at subunit-subunit interfaces
- O_2 binding to a "T" Hb subunit induces a conformational change in the heme, reorienting the F helix, which translates to an overall conformational change in the Hb subunit
- In R hemoglobin (all four hemes occupied by O_2) the subunit conformations are all as above, and different subunit-subunit interactions stabilize the 4° structure
- Binding of DPG in the central cavity of T Hb stabilizes the T conformation, decreasing O_2 binding affinity
- Asp94 and His146 on a β subunit are close enough to raise the pKa of His146 so it can protonate at the lower pH of tissue, forming a stabilizing salt bridge in T Hb
- Carbamaylation of the N-terminal amino group of the α subunits converts a positively charged group to a negatively charged group, allowing formation of a stabilizing salt bridge to a (+) AA in T hemoglobin
- The above information has been deduced based on static structures of Hb without Hb bound (T) or with four O_2 bound per Hb (R)

Cooperative binding of O_2 to Hb

- O_2 binding to a "T" Hb subunit induces a conformational change in the heme, reorienting the F helix, which translates to an overall conformational change in the Hb subunit
- Presumably, this conformational change in one subunit reorients adjacent subunit(s) making it easier for O_2 to bind successive subunits
- Does the reorientation of the other subunit(s) involve immediate conformational changes (before O_2 binds to them) or does it just make O_2 bind with higher affinity (lower K_d) followed by T to R conformational changes in the subsequent subunits(s)?
- The conformational changes, and overall structures of Hb with 1-3 O_2 bound (induced by sequential O2 binding) may be different than the structures of "all T" and "all R" Hb
- The mechanism of O_2-induced cooperativity is not fully understood, but binding of successive O_2 molecules increases affinity for binding of successive O_2
- affinity $O_{2(1)}<O_{2(2)},O_{2(3)}O_{2(4)}$





Sickle cell anemia