**PHRM 836 September 1, 2015**

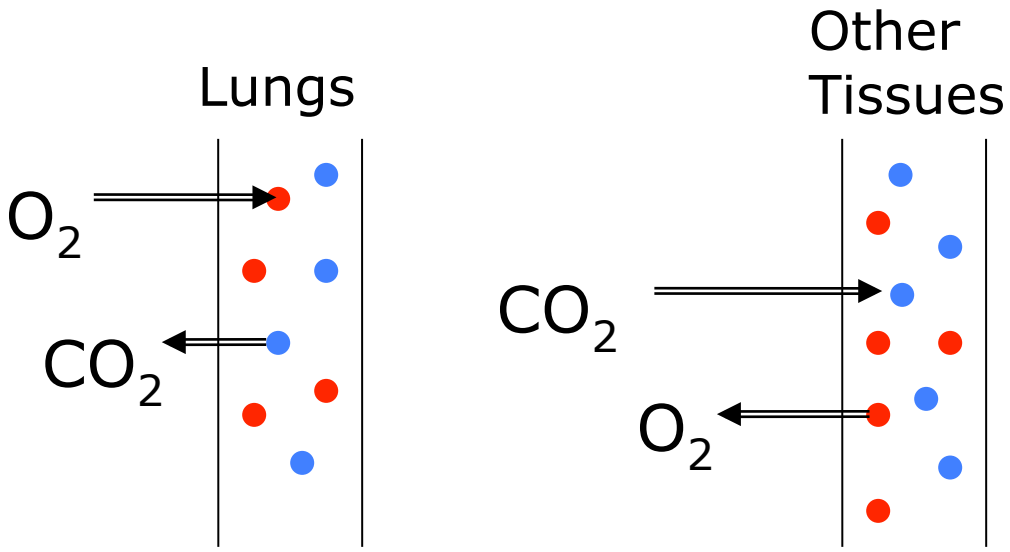
# Protein structure-function relationship: Allostery and cooperativity illustrated by hemoglobin (Hb) and myoglobin (Mb)

Devlin, section 9.4

1. Physiological role of Hb
2. Structure of Hb and comparison with Mb
3. Cooperativity of binding $O_2$
4. Regulation of $O_2$ binding

# Hemoglobin: physiological role = a transport protein

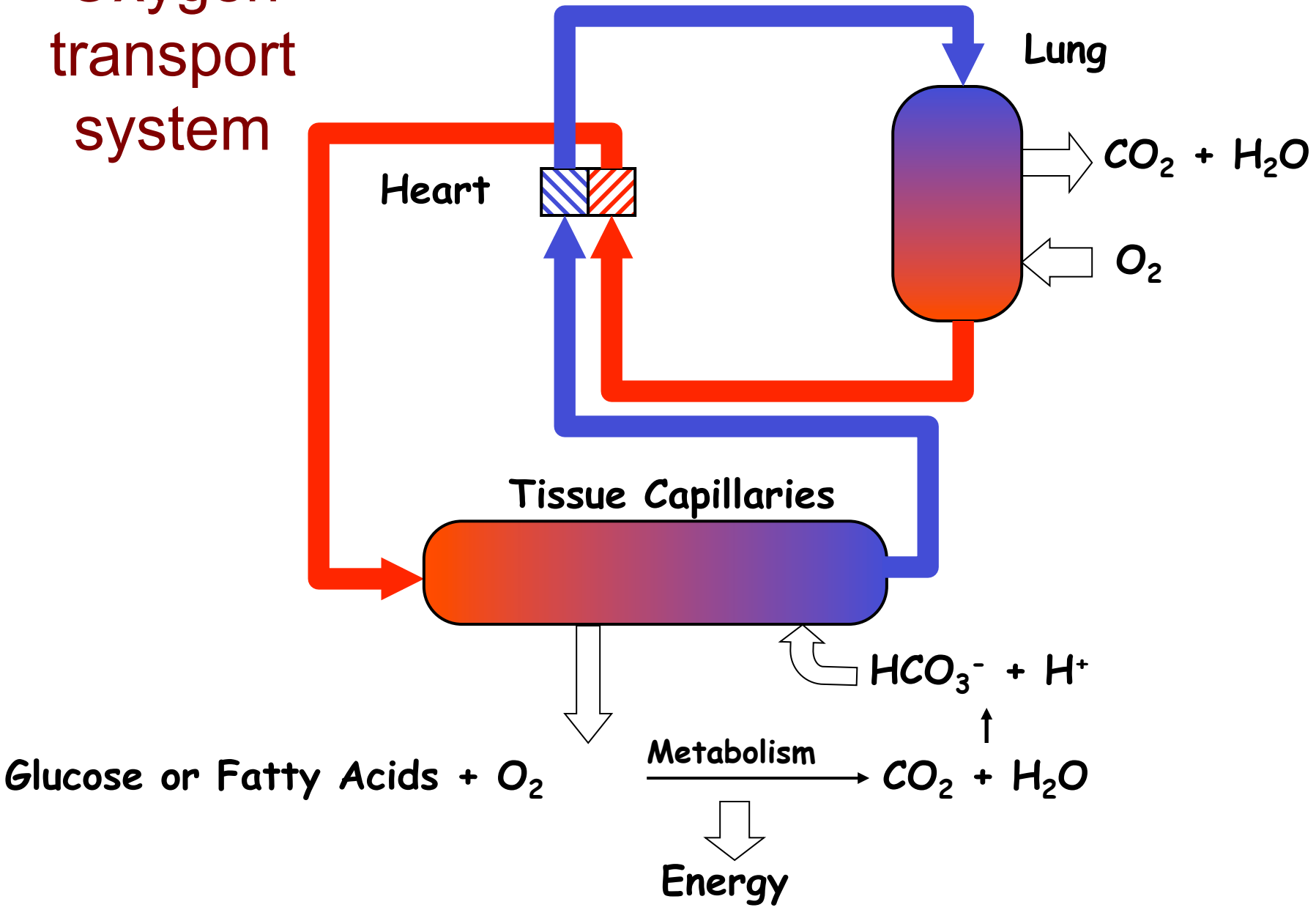
- Concentration in erythrocytes = $3 \times 10^8$ molecules/ cell. High concentration!
- Function: Transport $O_2$ and $CO_2$ between lung and tissues.





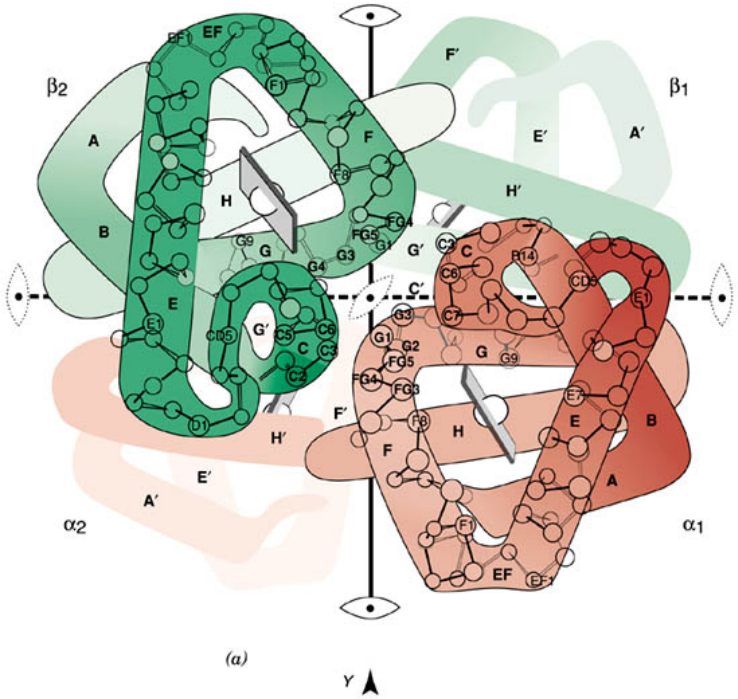
erythrocytes

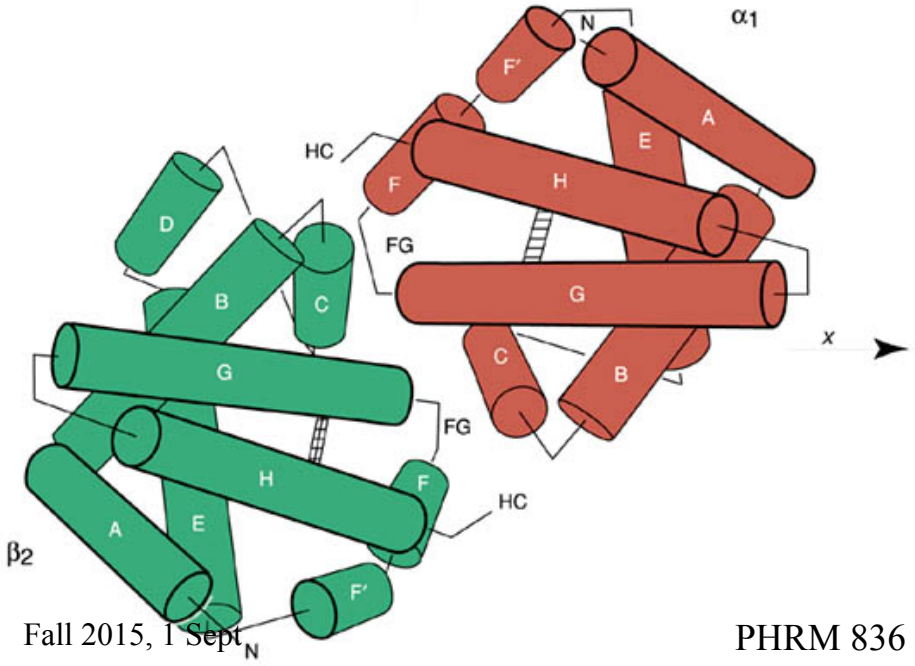
# Oxygen transport system



# Structure of Hemoglobin

- All-α protein:
  7-8 helices labelled A to H
- tetrameric: 2 x αβ dimer
- intersubunit interactions are critical for function



# Hemoglobin, a heme protein

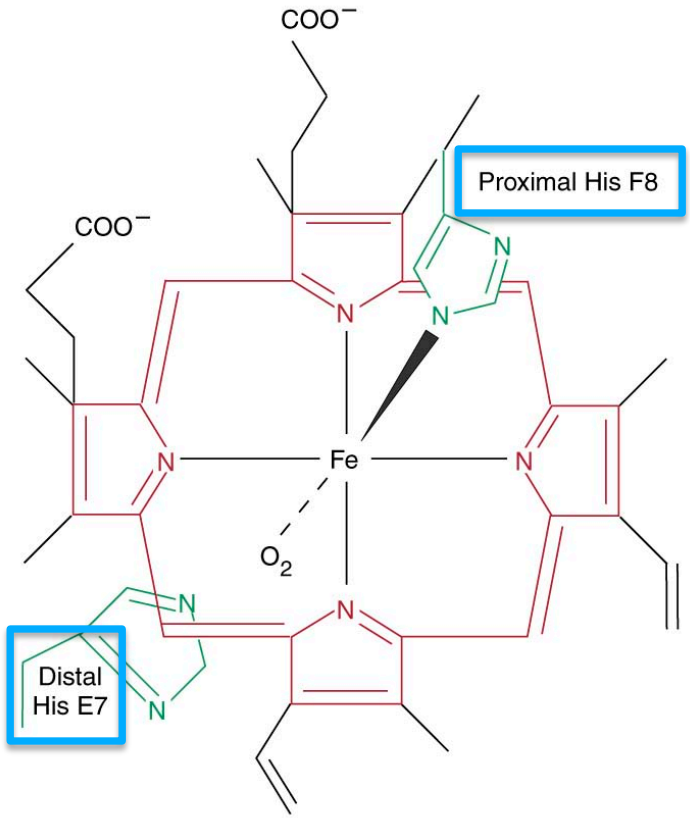
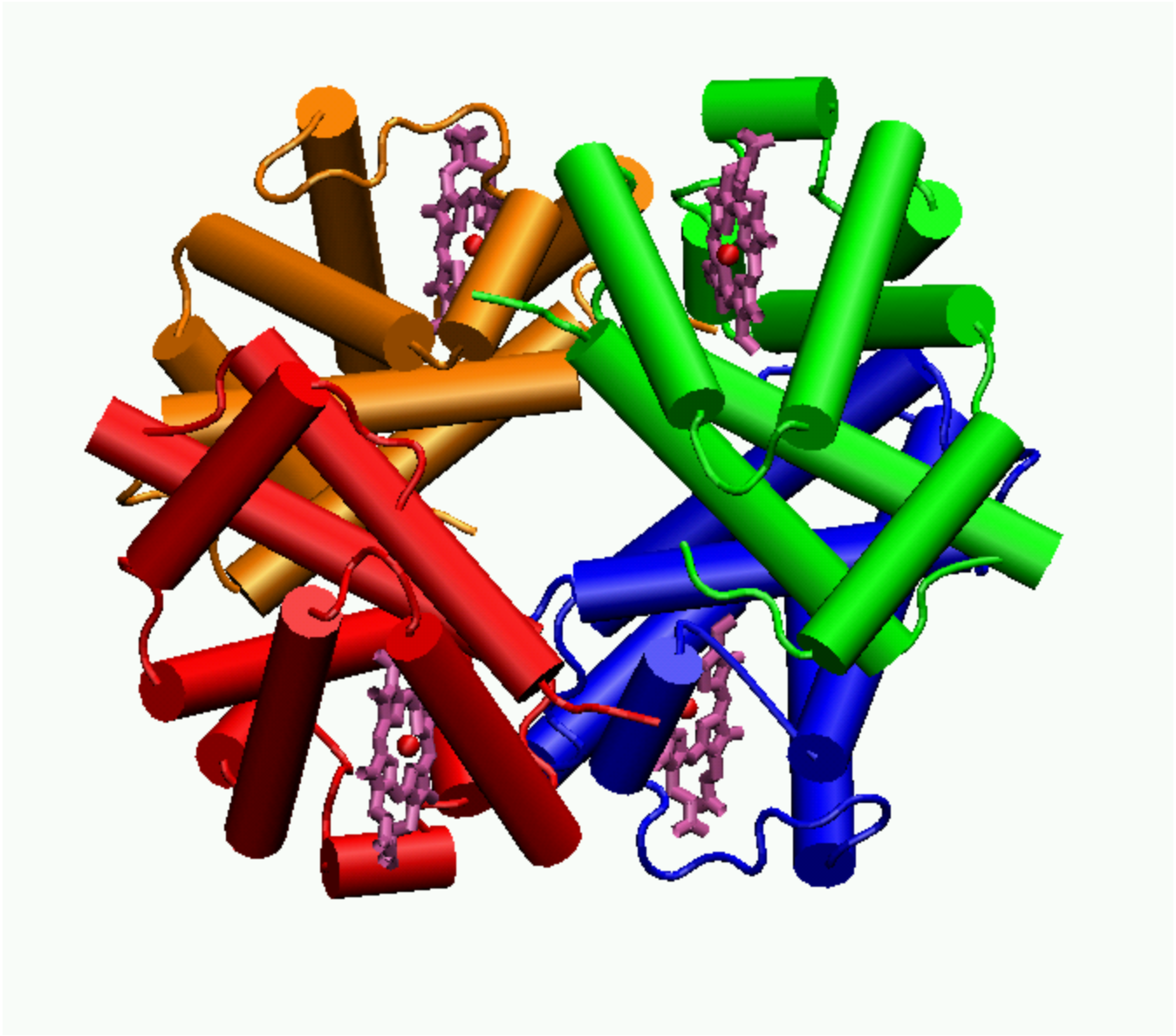



figure 9.21, Devlin

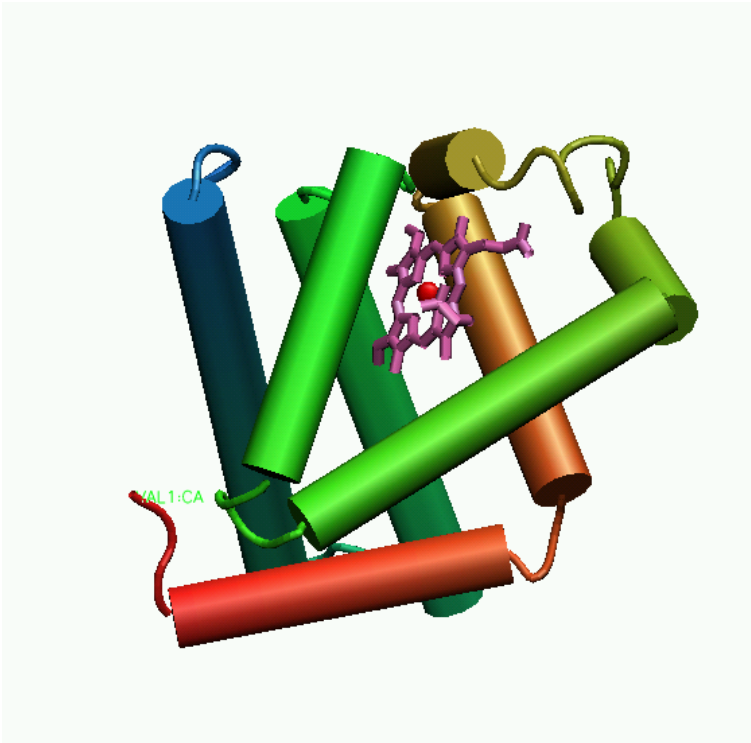
- Prosthetic group
  - ➔ Organic molecule needed for activity
  - ➔ Apoprotein = no prosthetic group
  - ➔ Holoprotein = + prosthetic grp
- Heme
  - ➔ Binds Fe
  - ➔ $O_2$ coordinates Fe of heme
  - ➔ Causes red color
  - ➔ Noncovalent association to Hb

# Tetrameric Hb

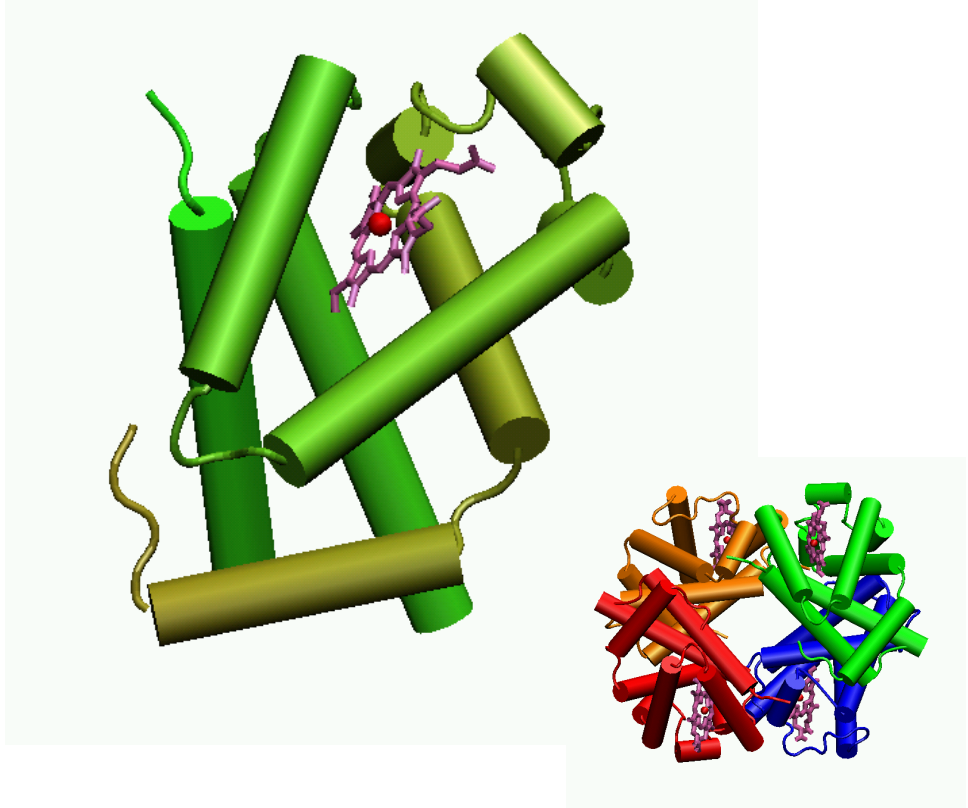


# Comparison with myoglobin (Mb)

- Structure: single chain, also with heme.
  - ➔ A monomeric "version" of hemoglobin
- Function: to store $O_2$ in muscle tissue.




Myoglobin



β chain of Hb

# Comparison of Hemoglobin And Myoglobin

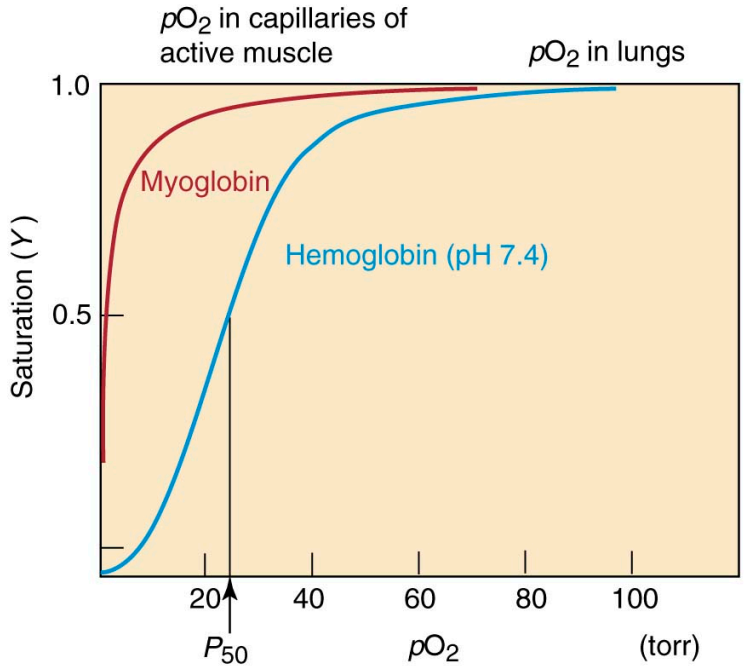
| **Features** | **Hemoglobin** | **Myoglobin** |
|---|---|---|
| No. of Polypeptide | 4 (2$\alpha$, 2$\beta$) | 1 |
| No. of Oxygen Bound | 4 | 1 |
| Amino Acids | 141 x 2<br>146 x 2 | 153 residues |
| Sites for transport | All cells | Skeletal muscle cells |
| Transport molecules | $O_2$, $CO_2$, NO | $O_2$ |

# $O_2$ Binding Curves

Fractional saturation of heme plotted against partial pressure of oxygen, $pO_2$.

$$Y = \frac{[XO_2]}{[XO_2]+[X]}, \; X = \text{Mb or Hb}$$

- Mb binding $O_2$ : single binding site; hyperbolic saturation curve
- Hb binding $O_2$: 4 binding sites; sigmoidal binding curve indicates cooperativity, *i.e.* binding to one site alters affinity of subsequent binding




50% of heme sites have $O_2$ bound

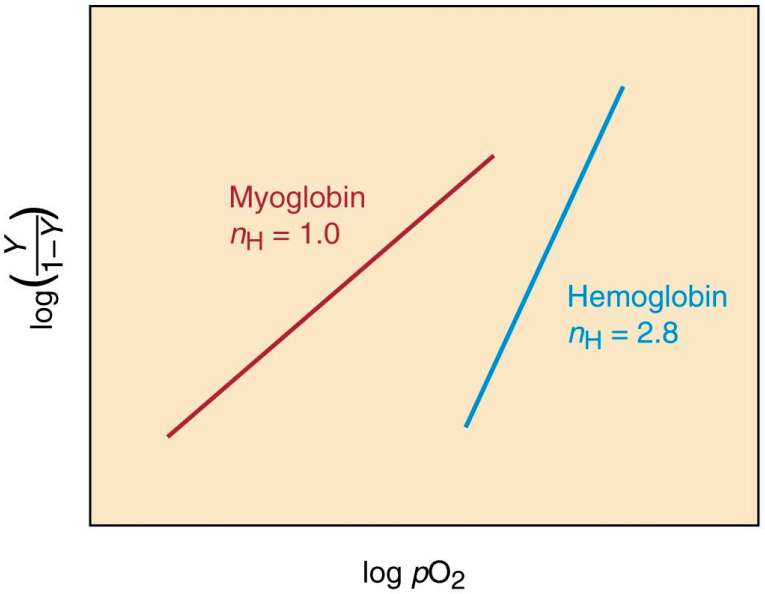
$P_{50}$ indicates $O_2$ affinity (see eqn 9.3, Devlin). Why??

# $O_2$ Binding Curves

Cooperativity for ligand binding

- Binding at one site (i.e. heme for Hb) facilitates binding to the second site.
- Positive cooperativity is an increase in binding affinity for each $O_2$ bound.
- <u>Hill equation & coefficient:</u> $n_H$:
  - ➔ determined from the slope of a log-log plot
  - ➔ measures the degree of cooperativity

$$\log\left(\frac{Y}{1-Y}\right) = \text{const} + n\log\left(pO_2\right)$$




n=1 no cooperativity

n>1 positive cooperativity

Fig 9.25, Devlin 7e

# $O_2$ Binding Curves

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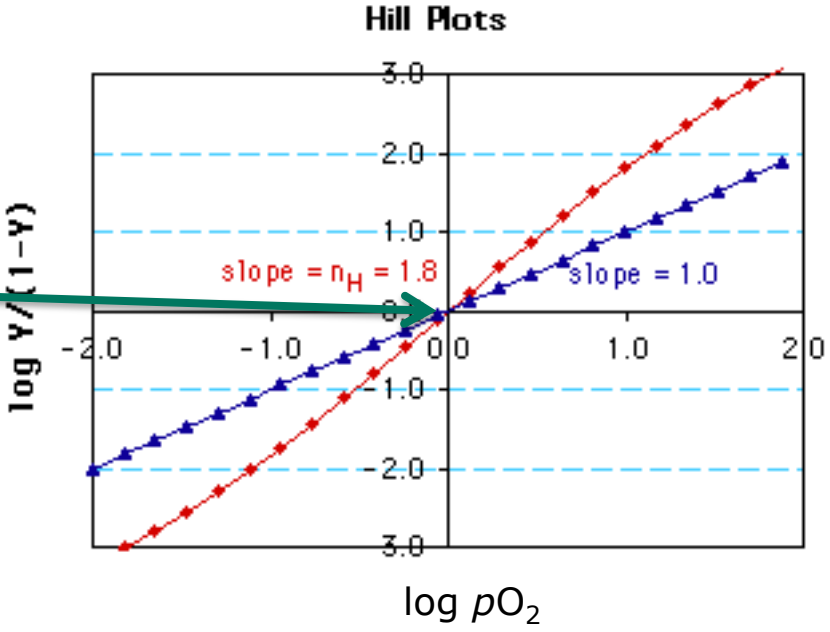
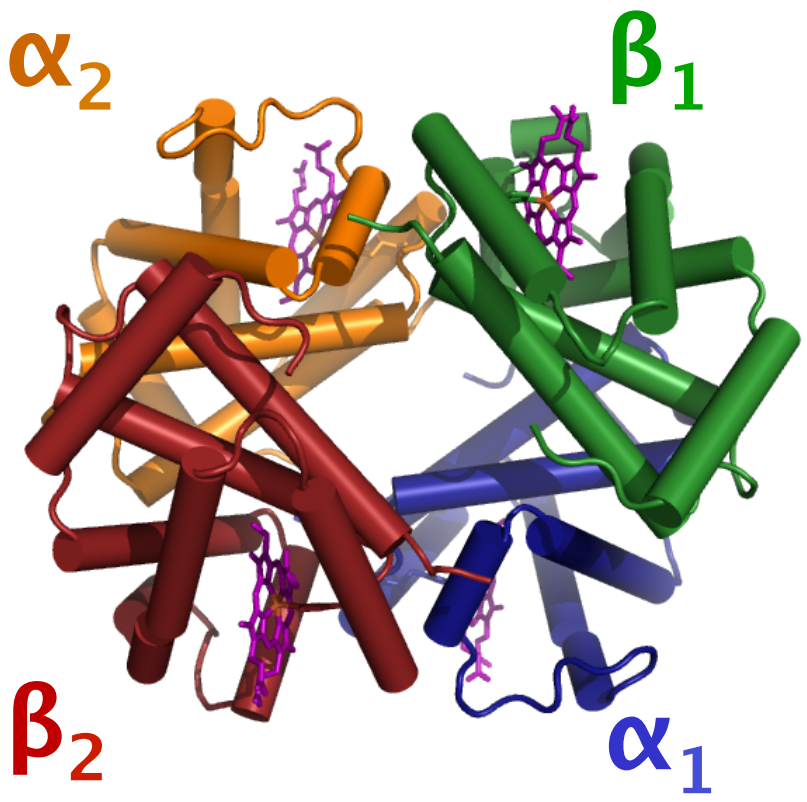



Slope at 50% saturation is equal to the Hill coefficient

# Structural Basis of Hb cooperativity

Crystallography shows oxyhemoglobin and deoxyhemoglobin differ in quaternary structure.

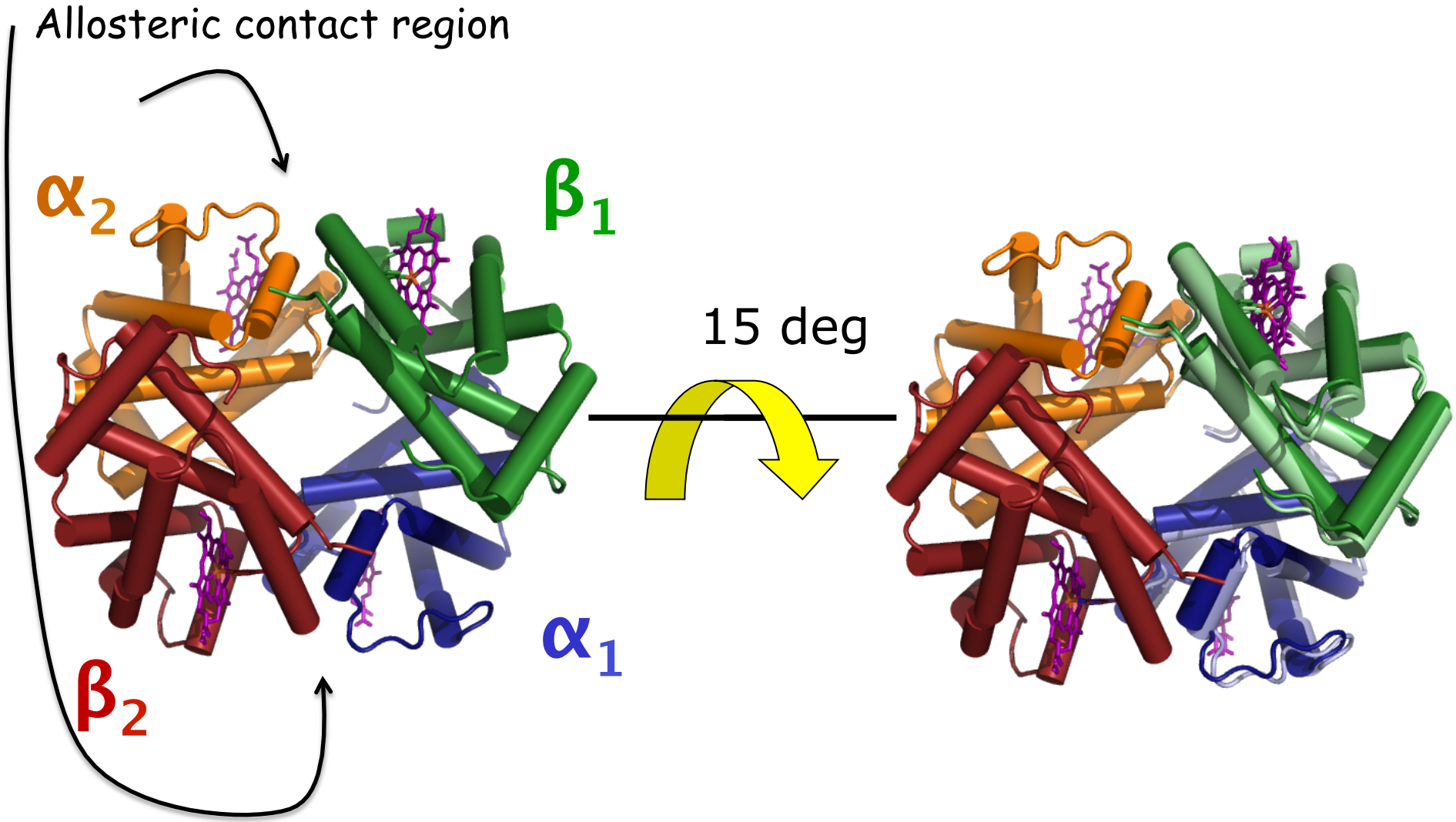
- One αβ dimer is rotated relative to the second dimer.
- Changes interactions at dimer interface between $\alpha_1\beta_1$ and $\alpha_2\beta_2$



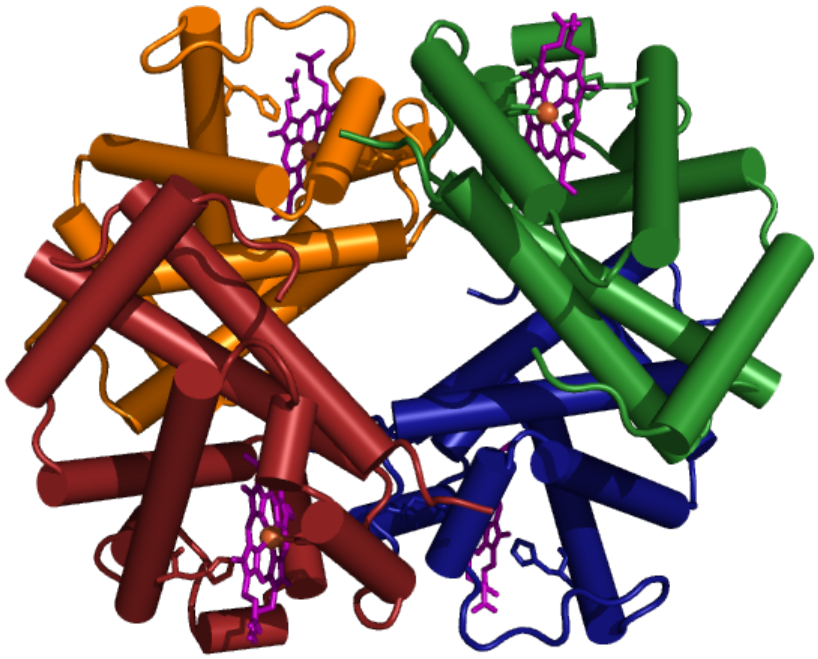

oxy-Hb is R state;

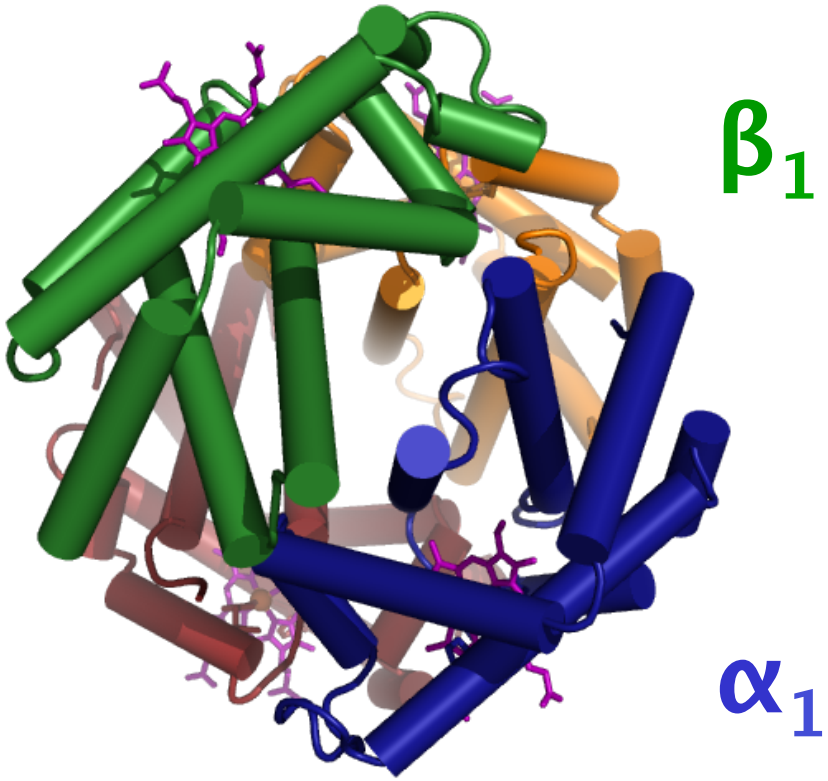
deoxy-Hb is T state

# $O_2$ binding changes the quarternary structure of Hb



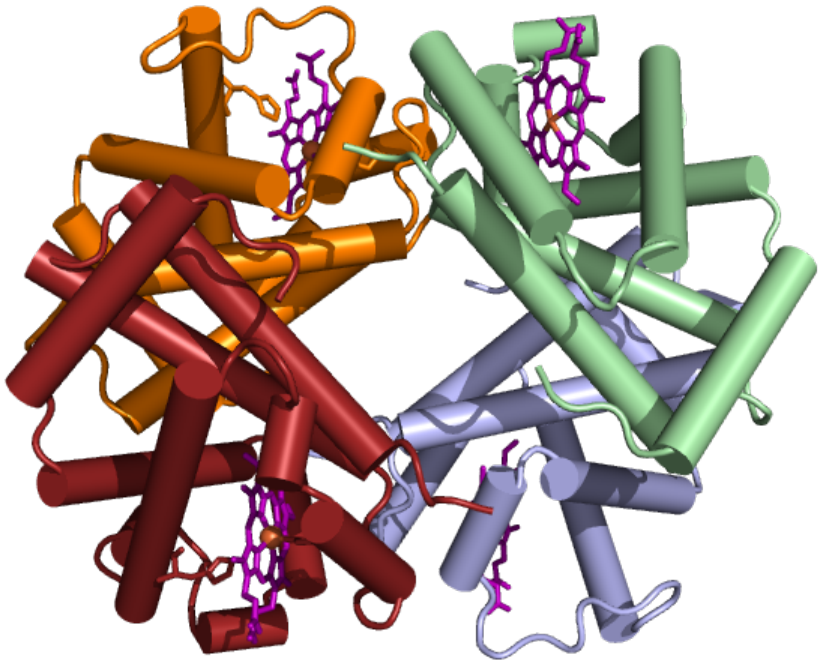
# $O_2$ binding changes the quarternary structure of Hb

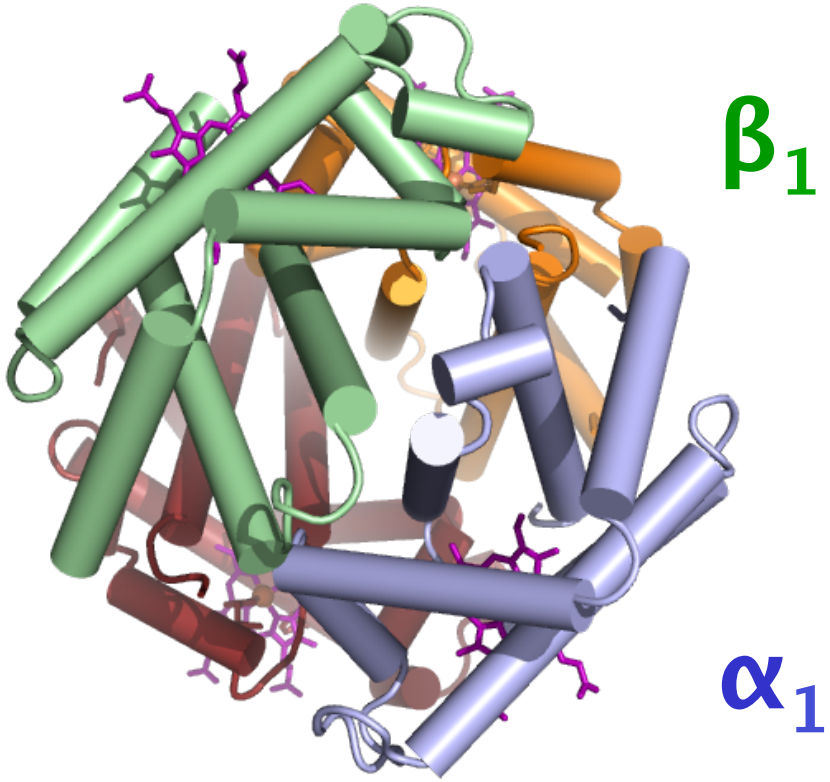





Different view from~90° rotation about an axis in the plane of the slide

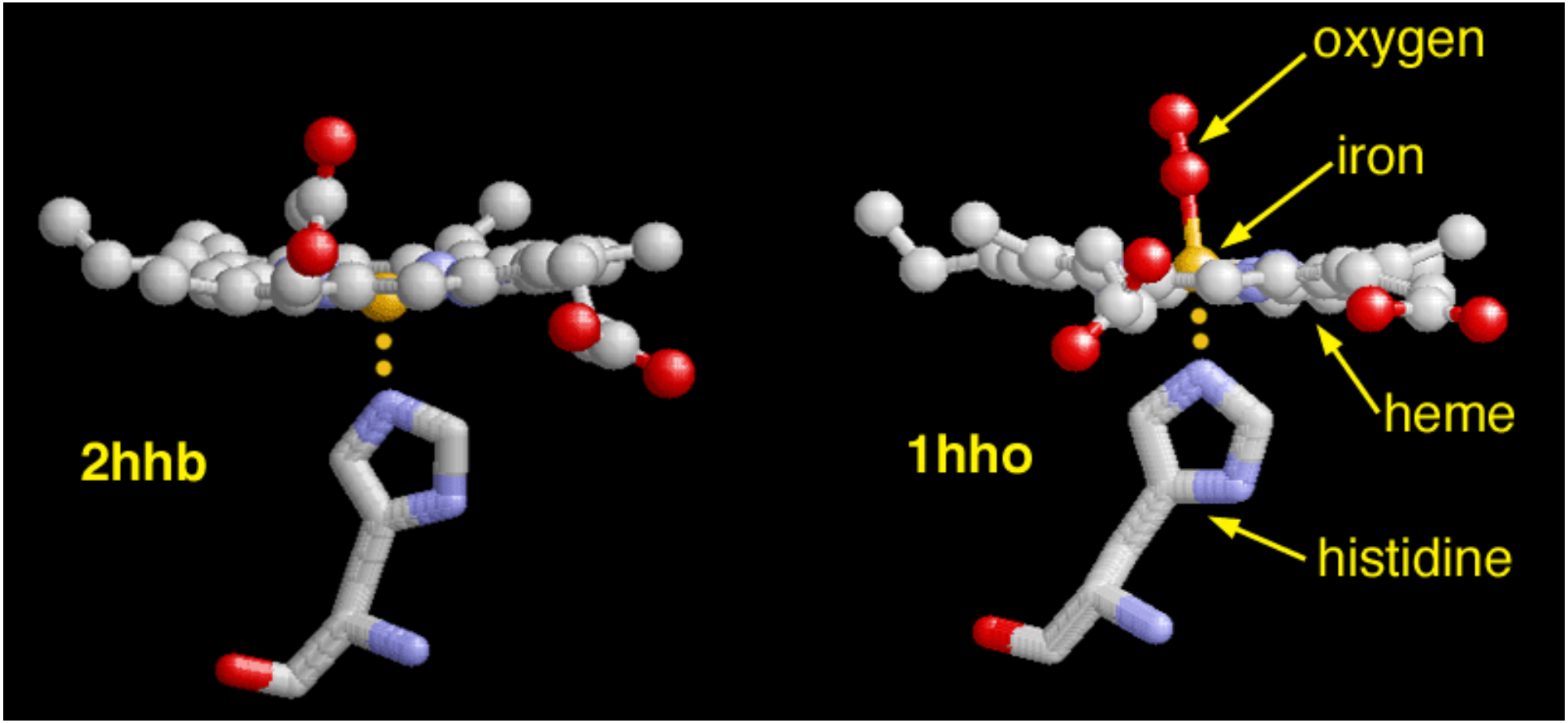
# $O_2$ binding changes the quarternary structure of Hb






Different view from~90° rotation about an axis in the plane of the slide

# Quarternary Change: how does $O_2$ binding induces it

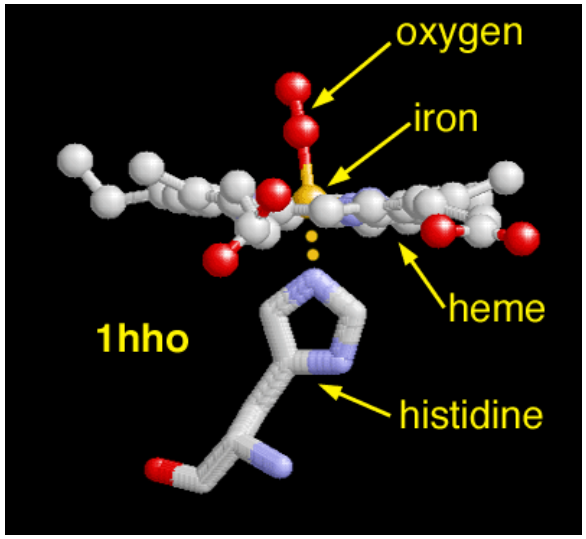



deoxy Hb: Fe has only 5 ligands and is out-of-plane

oxy Hb: Fe has only 6 ligands and is in-plane

# Quarternary Change

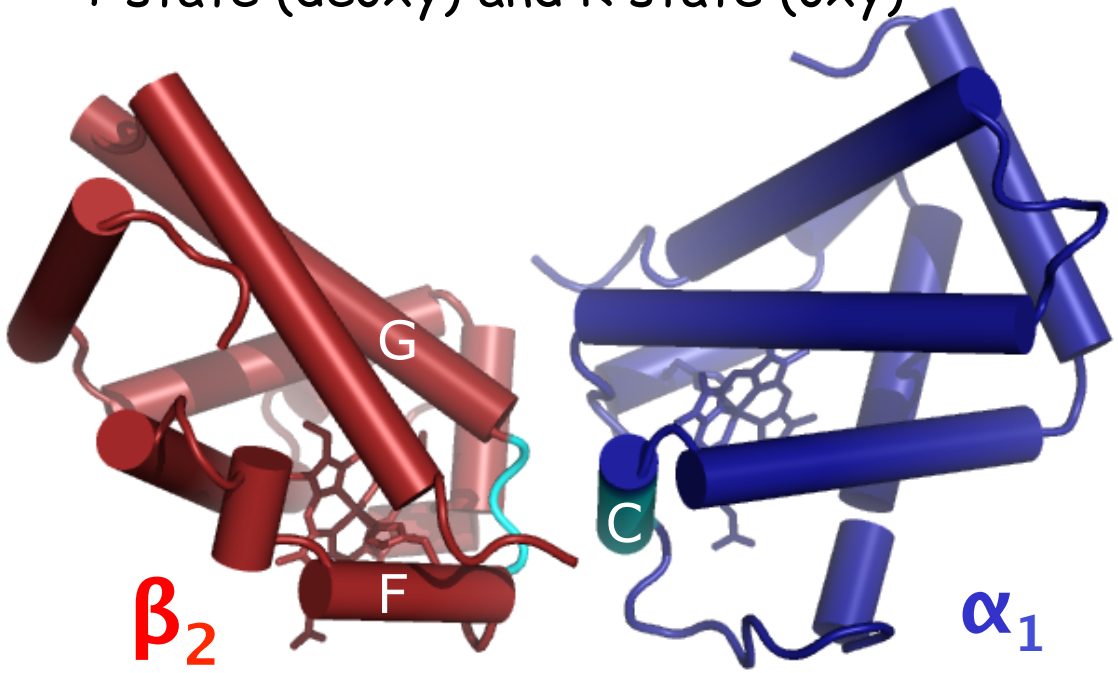
## triggered by oxygen binding that allows Fe to move into heme plane

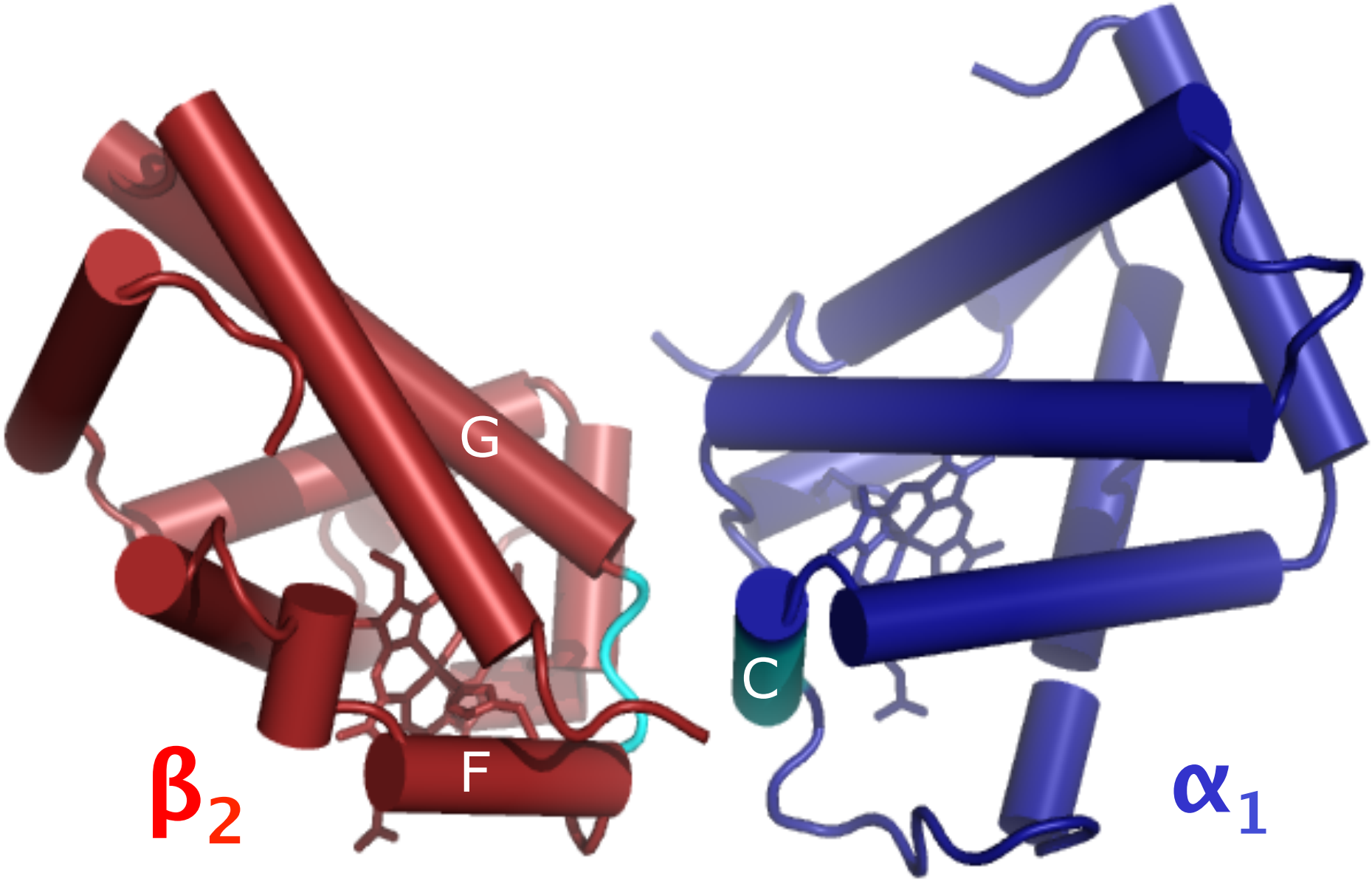



- drags proximal His in helix F and moves helix F
- this alters FG loop (of one chain) – C helix (another chain) interactions
- changes are propogated to neighboring heme and increase affinity
- T state (deoxy) and R state (oxy)

oxy Hb: Fe in plane alters proximal His

Analogous to
Fig 9.38, Devlin




G
F
C
β2
α1

# Bohr Effect

Important in metabolizing tissues: $CO_2$ produced; leads to lower pH; reduces Hb affinity for $O_2$.

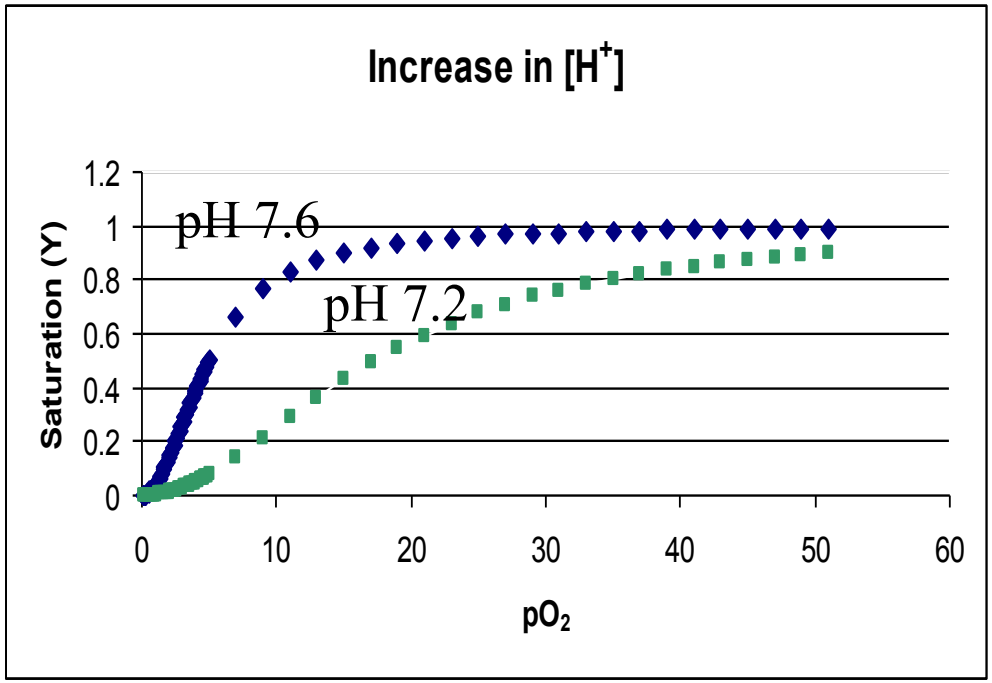
- Additional <u>regulation</u> of oxygen binding is from the Bohr Effect
  - ➔ Increase in [H+] (lower pH) decreases oxygen affinity, i.e. stabilizes deoxy Hb

$$Hb^T + 4O_2 \rightleftharpoons Hb^R(O_2)_4 + nH^+$$

*increase*

*drives equil to left*

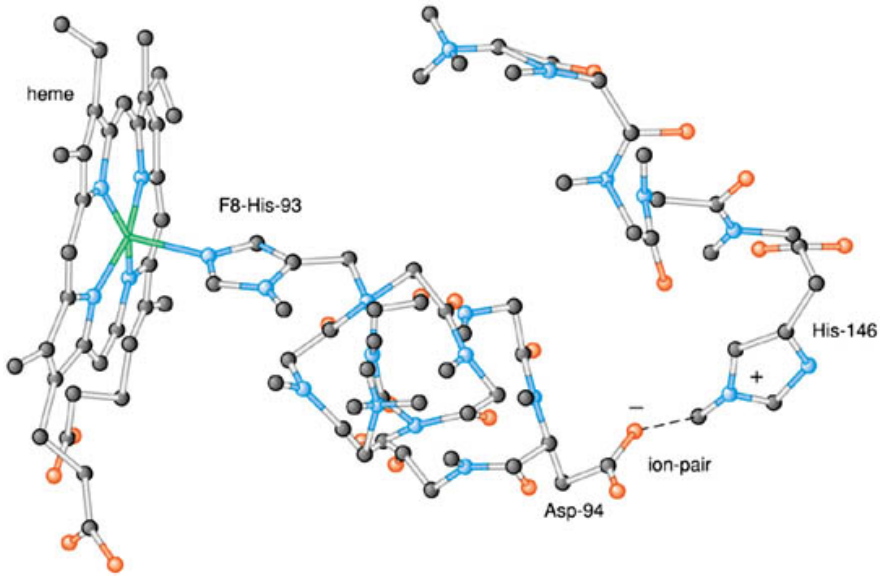
by law of mass action



# Structural basis of Bohr effect

- In deoxy-Hb, H146 forms salt bridge with D94.
- In oxy-Hb, dimer rotation disrupts this interaction and $H^+$ is released:
  - ➔ pKa in deoxyHb > pKa in oxyHb

$$Hb^T + 4O_2 \rightleftharpoons Hb^R(O_2)_4 + nH^+$$




Deoxy-Hb
Figure 9.30, Devlin

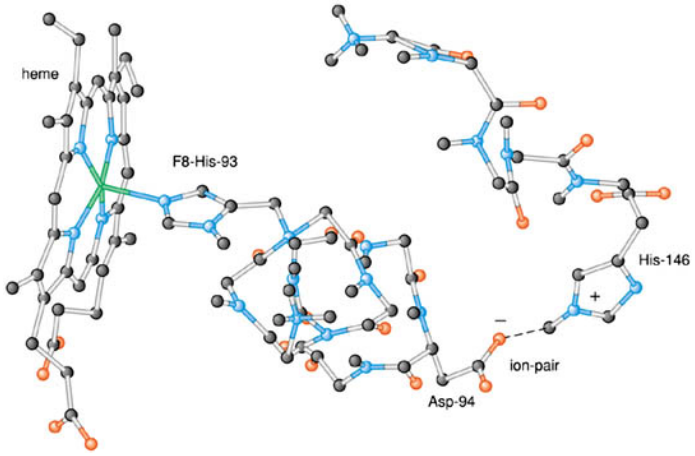
# Structural basis of Bohr effect

pH changes are coupled to carbon dioxide levels: in erythrocytes, carbonic acid dissociates to bicarbonate and a proton:

$$CO_2 + H_2O \underset{}{\overset{\text{carbonic anhydrase}}{\rightleftharpoons}} H_2CO_3 \overset{\text{spontaneous}}{\rightleftharpoons} HCO_3^- + H^+$$

$CO_2$ produced in tissues is converted to $HCO^-_3$ which increases $[H^+]$ (*i.e.* decreases pH) and promotes deoxy form/oxygen release.

$HCO^-_3$ is <u>transported in plasma</u> to lung, a process called isohydric transport, which accounts for ~80% of $CO_2$ transport to lungs.




Deoxy-Hb
Figure 9.30, Devlin

# Another Hb regulatory mechanism

## 2,3 bisphosphoglycerate (BPG)

- Allosteric effector: binds at a site different from $O_2$ and modulates Hb function. *Check out fig in Devlin*
- Effect is to change equilibrium for $O_2$ binding by lowering affinity, i.e. increasing $P_{50}$.
- binds to one form of Hb (deoxy-HB, not oxy-HB)
- Purpose: regulate binding under oxygen deficiency conditions. (Changes in BPG concentration occur over hours or days.)

$$Hb^T(BPG) + 4O_2 \rightleftharpoons Hb^R(O_2)_4 + BPG$$

*Hb was one of first targets for allosteric drugs*

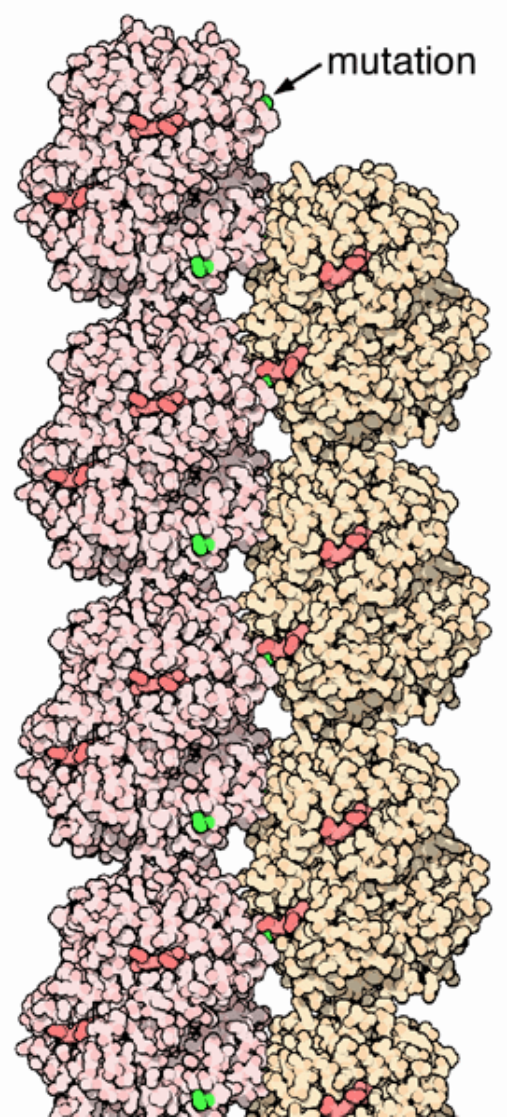
# Hb Variants

- Over 800 mutant Hb have been characterized
- Most are single amino acid substitutions
  - Surface residues – usually innocuous
  - Internal residues – destabilize the folded structure; carriers suffer from hemolytic anemia
  - Residues in heme binding pocket – eliminate binding of $O_2$
  - Changes in the α1β2 interface – changes in cooperativity. If oxy form is stabilized then release in tissues is less than normal. T form could also be stabilized.
- HbS, sickle-cell anemia
  - Substitution of β6 Glu, surface residue, by Val
  - Hb concentration is extremely high in red blood cells, nearly as dense as in a crystal
  - E to V changes the surface and causes inter-deoxyHb binding, which leads to polymerization in RBCs

# HbS, sickle-cell anemia

Glu 6 in the beta chain is mutated to valine. This change allows the deoxygenated form of the hemoglobin to stick to each other, as seen in PDB entry 2hbs

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



# *Summary of hemoglobin and cooperativity*

1. Hb transport function meets the physiological need to bind $O_2$ in lungs but release $O_2$ in tissue through several processes that affect the equilibrium between deoxy $Hb^T$ and oxy $Hb^R$ $(O_2)_4$
2. Positive cooperativity of $O_2$ binding derives from conformational changes, which propagate from the heme to certain tetrameric interfaces, and increases $O_2$ affinity
3. $O_2$ binding Hb is linked to $CO_2$ generation in tissues through pH (Bohr effect). This linkage leads to $CO_2$ transport opposite in direction to that of $O_2$.
4. $O_2$ binding affinity depends also on BPG concentration; BPG binds deoxy Hb and thus promotes release of $O_2$.