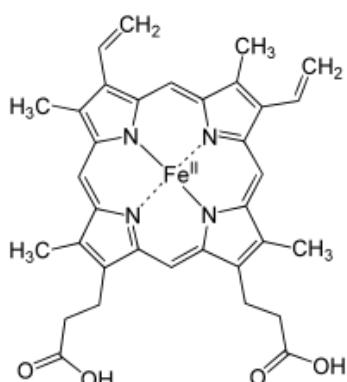




# Myoglobin & Haemoglobin

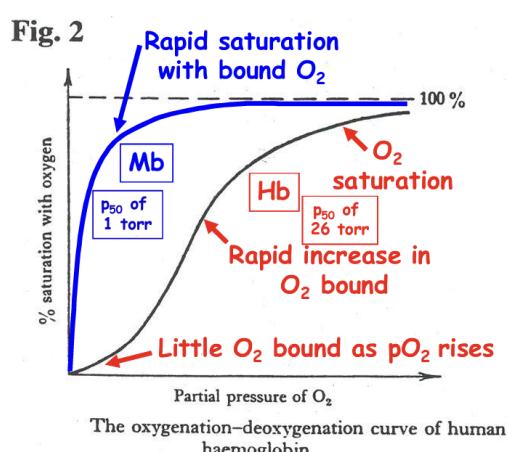
Course	Essential Protein Structure and Function
Confidence	Confident
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## Physiology of myoglobin and hemoglobin and its effectors



- Myoglobin and hemoglobin have a haem group that binds O<sub>2</sub>
- O<sub>2</sub> binds reversibly to Fe(II), not Fe (III)
  - Other five coordination sites are four N atoms of haem and a histidine
- Two carboxyl group makes hydrophilic contact with water outside the protein

## The oxygenation curve for Mb and Hb

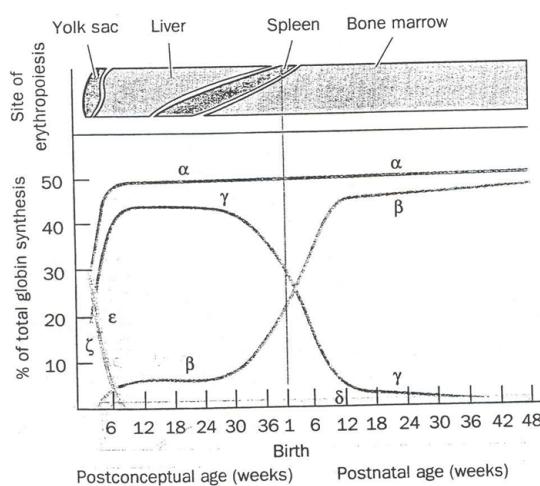


- Myoglobin shows a hyperbolic curve
  - Myoglobin has 1 protein subunit (1 haem) with simple reaction with oxygen
- Hb curve appears to be sigmoidal
  - Has 4 protein subunits
  - Low affinity to oxygen <~26 torr
- P50 is the O<sub>2</sub> pressure when the protein is 50% saturated with O<sub>2</sub>

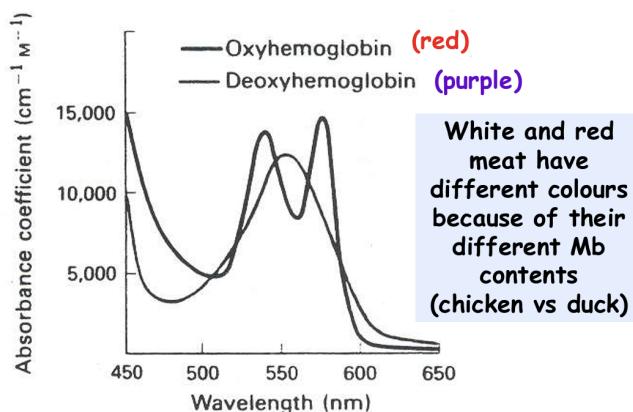
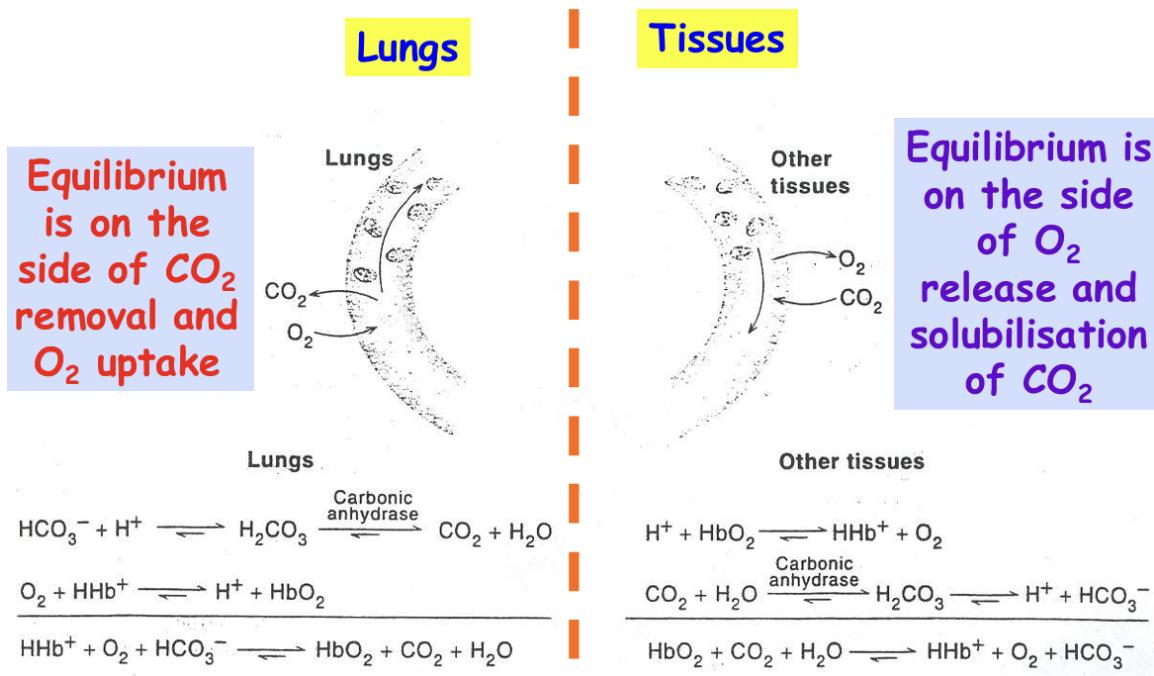
- Mb has high affinity for oxygen and rapid saturation

## Progression of human globin chain synthesis with embryonic and fetal development

- Note that any RBC contains only one type of each alpha- and beta-like subunits.

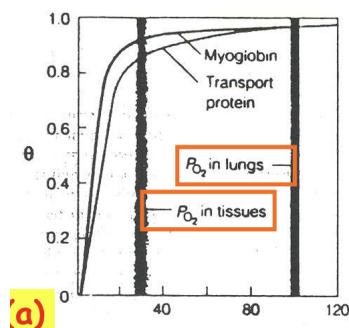


- Fetal Hb:  $\alpha_2\gamma_2$
- Adult Hb:  $\alpha_2\beta_2$ 
  - *The gamma chain, a result of gene duplication, is 72% identical in amino acid sequence with the b chain.*
- The progression in the sites of erythropoiesis (red cell formation) corresponds roughly to the major switches in Hb types
- The fetal Hb has higher oxygen affinity so it can be delivered to the fetus
- Hemoglobin circulates in the blood
  - It picks up O<sub>2</sub> and release CO<sub>2</sub> in the lungs
  - It picks up CO<sub>2</sub> and release O<sub>2</sub> in the tissues
- Myoglobin is an O<sub>2</sub> store found in all tissues
- Chemical reactions - note both O<sub>2</sub> and CO<sub>2</sub> are being transported

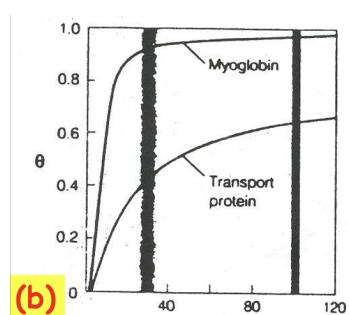


- Hb and Mb have very similar absorption spectra
- Visible absorption spectra change markedly on binding oxygen
- White and red meat have different colours because of their different Mb contents
- Lips turn blue because of lack of oxygen
- Chemical basis of monitoring blood oxygen level
  - Pulse oximetry is a non-invasive method that measures the oxygen saturation of hemoglobin in arterial blood by analyzing the absorption of light at specific wavelengths.

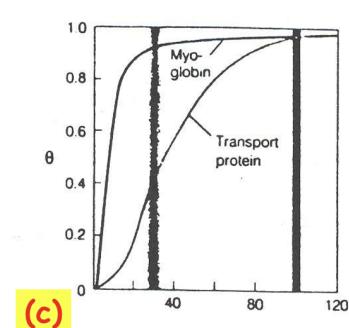
## Why do we need a sigmoidal O<sub>2</sub> binding curve?



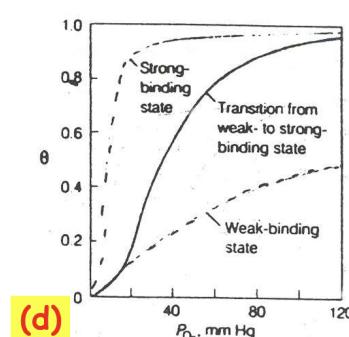
- A hyperbolic high affinity Mb-type transporter will not unload O<sub>2</sub> to Mb in the tissues
  - P<sub>O<sub>2</sub></sub> in the lungs = 100 torr
  - P<sub>O<sub>2</sub></sub> in the tissue = ~30 torr
- Not enough difference between affinity in the lungs and tissues



- A hyperbolic low affinity Mb-type transporter will not pick up enough O<sub>2</sub> in the lungs



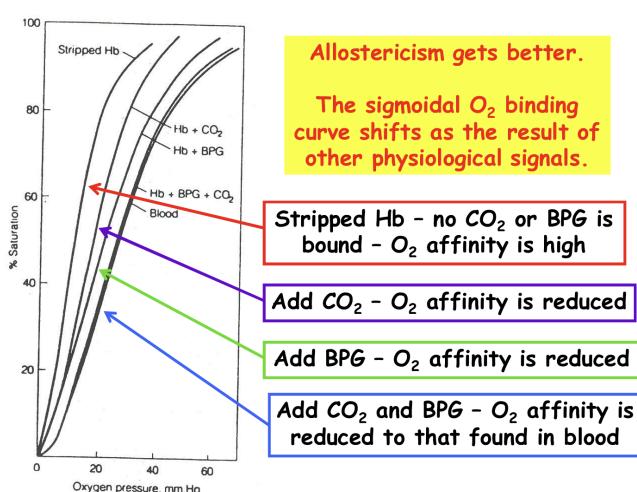
- A sigmoidal curve satisfies both criteria of low affinity in the tissues and high affinity in the lungs
  - Give up O<sub>2</sub> in the tissues and pick up in the lungs
  - Maximise efficiency in transporting oxygen



- A sigmoidal curve results from a transition between a weak-binding state and a strong-binding state.
  - This is allostericism
  - Weak binding state - T state
  - Strong binding state - R state

## Haemoglobin is an allosteric transporter

- **Allosteric proteins** - the binding of one ligand causes the affinity for binding other copies of the ligand the change (include enzymes)
- The binding of the first O<sub>2</sub> molecule to Hb increases the affinity of binding more O<sub>2</sub>



- An allosteric protein can be easily regulated and thus useful
  - Change the way which oxygen get transported in the blood
- The sigmoidal O<sub>2</sub> binding curve may shift as the result of other physiological signals

- Four allosteric effectors encourage Hb to drop its O<sub>2</sub> content - so these other ligands also affect O<sub>2</sub> binding
  - Oxygen
- ▼ Hydrogen ions (pH)
  - The regulation of oxygen binding by hydrogen ions and carbon dioxide is called the Bohr effect.
  - The oxygen affinity of hemoglobin decreases as pH decreases from a value of 7.4. Consequently, as hemoglobin moves into a region of lower pH, its tendency to release oxygen increases.
  - In deoxyhemoglobin, the terminal carboxylate group of b 146 forms a ionic bond, also called a salt bridge, with a lysine residue in the a subunit of the other ab dimer. This interaction locks the side chain of histidine b 146 in a position from which it can participate in a salt bridge with negatively charged aspartate b 94 in the same chain, provided that the imidazole group of the histidine residue is protonated
  - In addition to His b 146, the a -amino groups at the amino termini of the a chain and the side chain of histidine a 122 also participate in

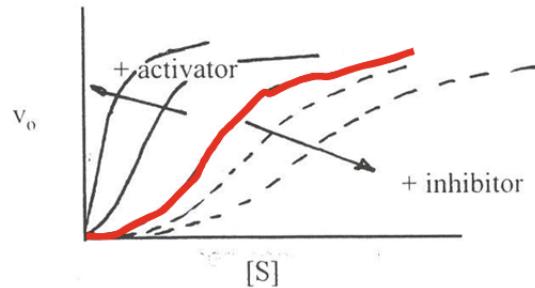
salt bridges in the T state. The formation of these salt bridges stabilizes the T state, leading to a greater tendency for oxygen to be released.

#### ▼ Carbon dioxide

- First, the presence of high concentrations of carbon dioxide leads to a drop in pH within the red blood cell. Thus stabilising T state as hydrogen ions.
- Secondly, Carbon dioxide directly stabilizes deoxyhemoglobin by reacting with the terminal amino groups to form carbamate groups, which are negatively charged, in contrast with the neutral or positive charges on the free amino group. The amino termini lie at the interface between the ab dimers, and these negatively charged carbamate groups participate in salt-bridge interactions that stabilize the T state, favoring the release of oxygen.
  - Organic phosphate
    - BPG - biphosphoglycerate, produced during metabolism in the glycolytic pathway
- These bind more strongly to deoxy-Hb than to oxy-Hb, stabilising the T state, hence shifting the O<sub>2</sub> curve to the right

## Theories of co-operativity

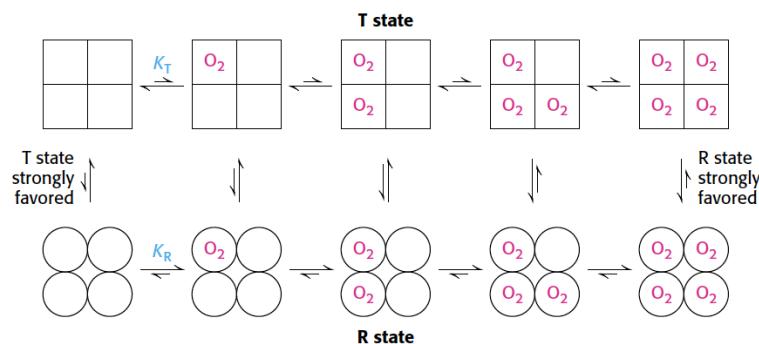
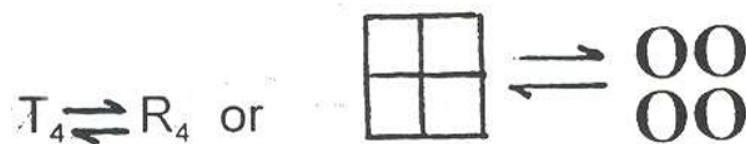
- Allosteric enzymes show co-operative substrate binding.
  - The degree of co-operativity is modulated by allosteric effectors.
  - An allosteric inhibitor increases co-operativity to slow down the rate at a specific [S].
  - An allosteric activator decreases co-operativity.
    - At high activator concentrations the v<sub>0</sub> vs [S] plot becomes hyperbolic.



- This behavior may be explained by the concerted model or the sequential model. Both require a quaternary structure.

### Concerted Model

- The protein can exist in two states, R and T, which are in equilibrium.
- T - taut state (deoxy-Hb); R - relaxed state (oxy-Hb)
- R has high affinity for substrate and T low affinity.
- All subunits must be in the same state.

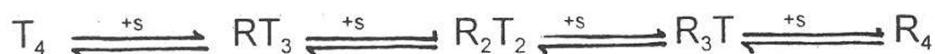


- When a substrate is added it binds preferentially to R<sub>4</sub>, pulling the equilibrium towards R<sub>4</sub>.
  - Thus, as a hemoglobin tetramer binds each oxygen molecule, the probability that the tetramer is in the R state increases.
- This gives the characteristic sigmoid curve as [S] is increased.

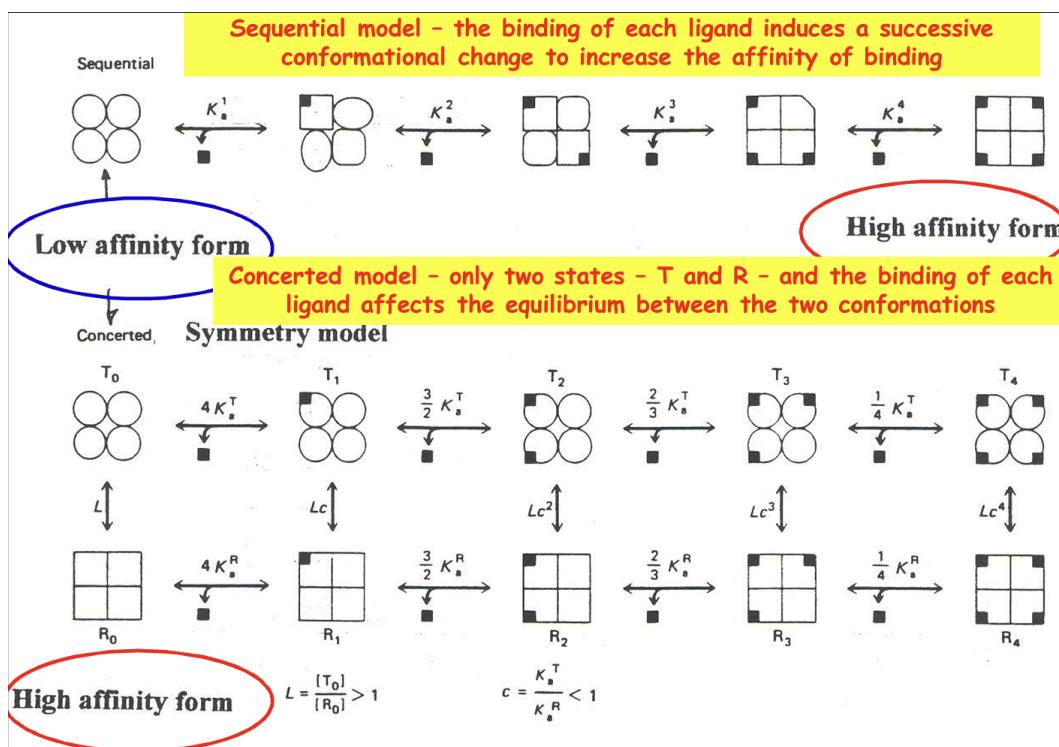
- An allosteric inhibitor increases co-operativity by binding and stabilising the T state; an activator binds to and stabilises R.
- This model fits the behavior of hemoglobin and aspartate transcarboxylase (ATCase)
- Note that “hybrid” states do not appear.

### Sequential Model

- The sigmoidal behavior is produced by the substrate binding to a subunit inducing the T to R transition in that subunit.
- *The binding of a ligand to one site in an assembly increases the binding affinity of neighboring sites without inducing a full conversion from the T into the R state*
  - The change in one subunit influences its neighbors



- Hybrid states must occur. Activators stabilise R state, inhibitors the T state.



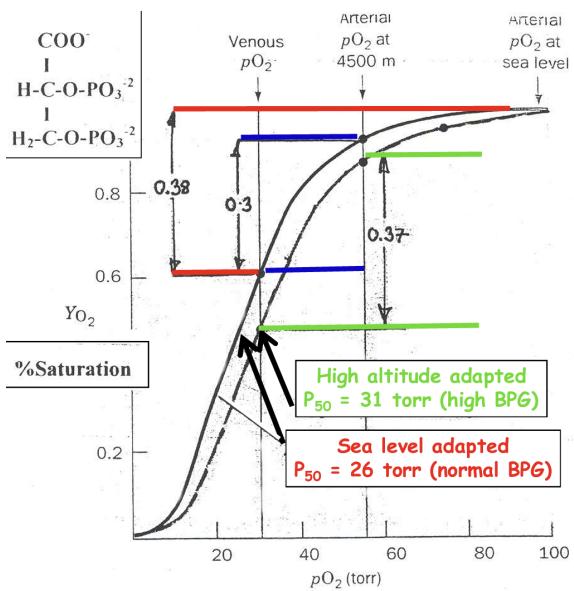
- A combined model is required.

- Hemoglobin behaviour is concerted in that the tetramer with three sites occupied by oxygen is almost always in the quaternary structure associated with the R state. The remaining open binding site has an affinity for oxygen more than 20-fold greater than that of fully deoxygenated haemoglobin binding its first oxygen.
- However, the behavior is not fully concerted, because haemoglobin with oxygen bound to only one of four sites remains primarily in the T-state quaternary structure. Yet, this molecule binds oxygen three times as strongly as does fully deoxygenated hemoglobin, an observation consistent only with a sequential model.
- These results highlight the fact that the concerted and sequential models represent idealized limiting cases, which real systems may approach but rarely attain.

## Four T-state ligands and one R-state ligand - Where do they bind in Hb

Table 1. <i>Allosteric Effectors of O<sub>2</sub> Affinity of Hemoglobin</i>			
These ligands promote	DECREASE AFFINITY	BINDING SITE ON HUMAN T STATE	
		α-Chain	β-Chain
T-State	Organic phosphates (e.g., diphosphoglycerate) <b>BPG</b>	α-NH <sub>3</sub> <sup>+</sup>	His 2
	CO <sub>2</sub>	α-NH <sub>2</sub>	Lys 82
	Anions (e.g., Cl <sup>-</sup> )	α-NH <sub>3</sub> <sup>+</sup>	His 143
R-State	H <sup>+</sup> (Bohr effect)	Arg 141	α-NH <sub>2</sub>
		α-NH <sub>2</sub>	Lys 82
			His 146
INCREASE AFFINITY			
O <sub>2</sub> and other heme ligands		BINDING SITE ON R STATE	
		α-Chain	β-Chain
		heme Fe	heme Fe

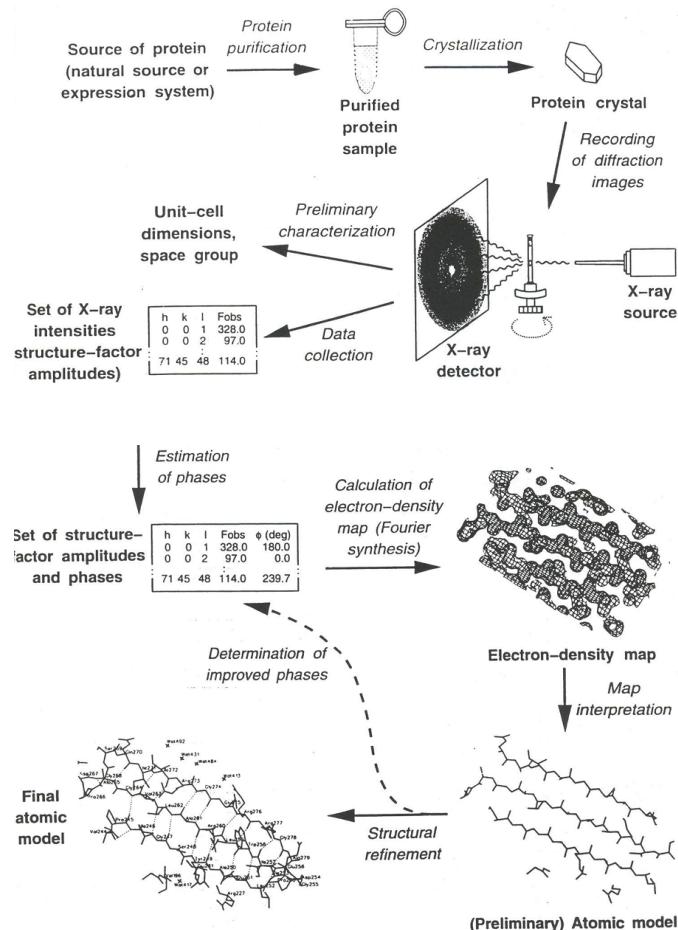
## The O<sub>2</sub>-dissociation curve of blood adapted to sea-level and high altitude



- Because of the change of oxygen intake, people might experience some dizziness, shortness of breath, headaches and nausea
- At sea level, the arterial pO<sub>2</sub> of 100 torr and venous pO<sub>2</sub> 30 torr means Hb unloads 38% of the O<sub>2</sub> it carries
- At an altitude of 4500 metres, the arterial pO<sub>2</sub> is 55 torr and Hb unloads 30% of the O<sub>2</sub> it carries.
- An increase in BPG levels shifts the curve to the right, so Hb can unload 37% of its load of O<sub>2</sub>.
- Left: the effect of high altitude exposure on the P<sub>50</sub> and the BPG concentration in blood in sea-level-adapted individuals. Two days to adapt
- Right: the effect of exposure to sea-levels on high-altitude adapted individuals

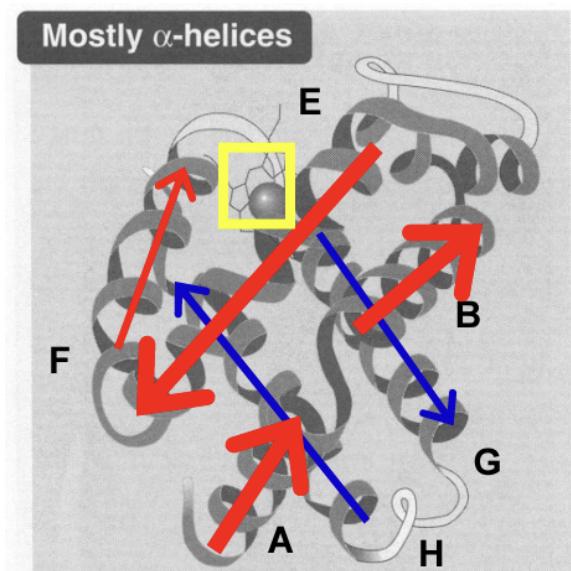
## Structures of Myoglobin and Hemoglobin and its different forms

### Structures from protein crystallography



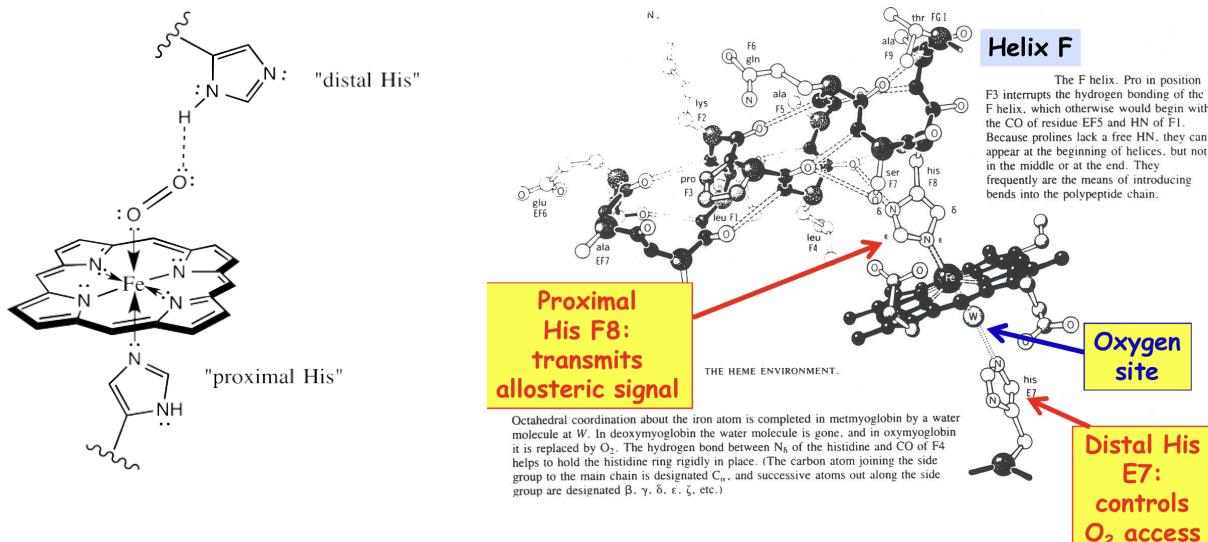
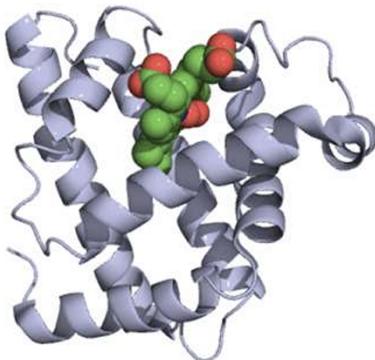
## Structure of myoglobin - one protein subunit

**Secondary structure:** The alpha-helices provide a buried heme pocket



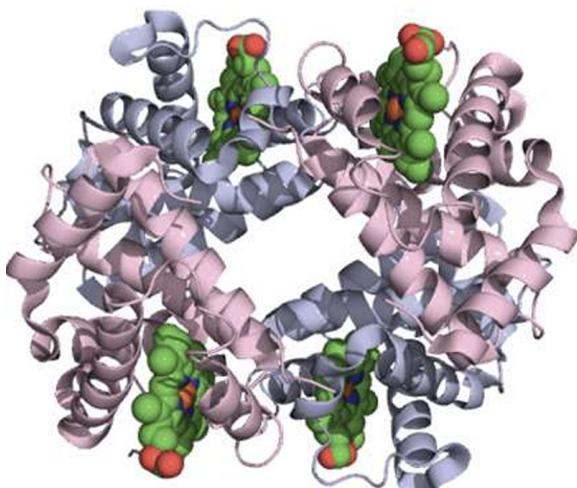
- Myoglobin and hemoglobin have 8 structurally similar alpha helices labelled A to H, making 75% of the secondary structure
  - $\alpha$ -helix: 131 amino acids, total: 153 amino acids
- Helices A, B, E are on the top, helix F passes from top to bottom, helices G, H are on the bottom
- The heme is sandwiched in the angle between helices E and F.

- The function of the  $\alpha$ -helices is to provide a pocket for the hydrophobic heme.
- Note the carboxyl group of the heme is outside, making contact with water



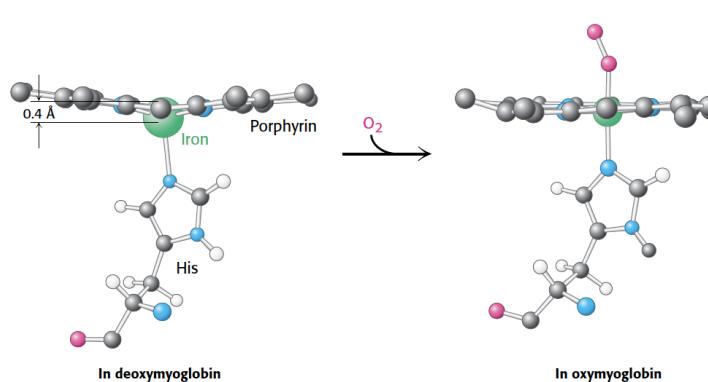
## Structure of hemoglobin

Four protein subunits (2 alpha and 2 beta) which associate as an  $\alpha_1\beta_2$  and an  $\alpha_2\beta_1$  dimer



- All four haem groups are at the surface, carboxyl group facing water
- No contact between the four hemes.
- Tetrahedral packing of the alpha and beta subunits

- The trigger for the allosteric conformational change: Oxygen binding changes the position of the iron ion.



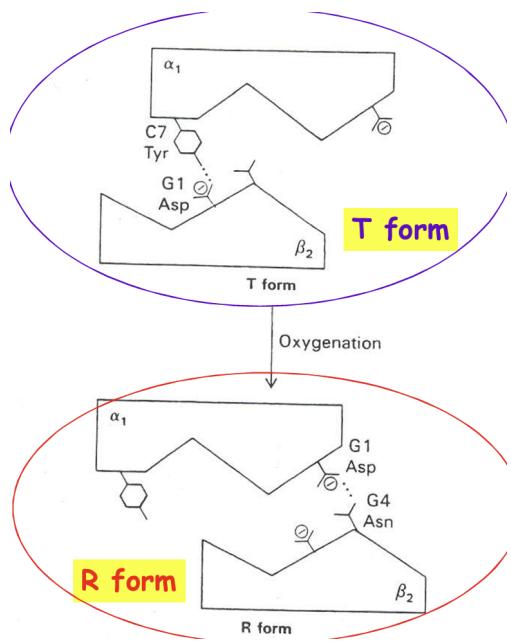
- Binding of O<sub>2</sub> to the Fe(II) pulls the Fe atom in to the plane of the haem.
- The Fe then pulls His F8 (proximal) and Helix F moves with this.

#### ▼ More detailed about Fe II

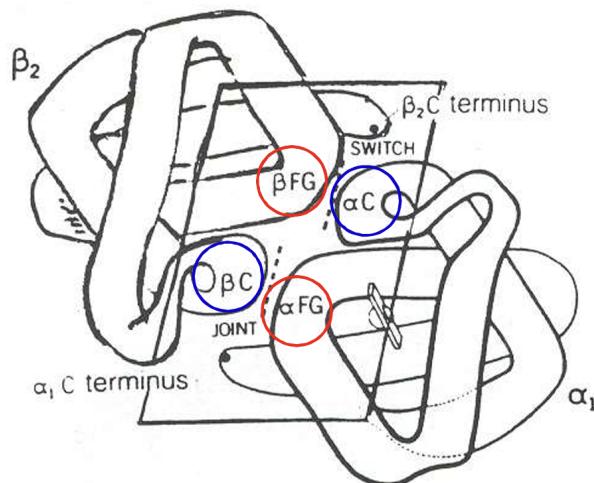
The iron atom lies in the center of the protoporphyrin, bonded to the four pyrrole nitrogen atoms. Although the heme-bound iron can be in either the ferrous (Fe 2) or ferric (Fe 3) oxidation state, only the Fe 2 state is capable of binding oxygen. The iron ion can form two additional bonds, one on each side of the heme plane. These binding sites are called the fifth and sixth coordination sites. In myoglobin, the fifth coordination site is occupied by the imidazole ring of a histidine residue from the protein. This histidine is referred to as the proximal histidine. Oxygen binding occurs at the sixth coordination site.

In deoxymyoglobin, this site remains unoccupied. The iron ion is slightly too large to fit into the well-defined hole within the porphyrin ring; it lies approximately 0.4 Å outside the porphyrin plane. Binding of the oxygen molecule at the sixth coordination site substantially rearranges the electrons within the iron so that the ion becomes effectively smaller, allowing it to move within the plane of the porphyrin.

### The switch between two allosteric conformations



Front view of hemoglobin showing extensive subunit interactions between the FG corners and C helices



- The  $\alpha_1\beta_2$  interface switches from the T to the R form on oxygenation.
- The dove-tailed construction of this interface allows the subunits to readily adopt either of the two conformations
- Important interaction regions from top to bottom are the  $\beta$ -chain termini, switch region, flexible joint and  $\alpha$ -chain termini

### BPG binds to the central cavity of T state

#### ▼ Detailed about BPG

For hemoglobin to function efficiently, the T state must remain stable until the binding of sufficient oxygen has converted it into the R state. In fact, however, the T state of hemoglobin is highly unstable, pushing the equilibrium so far toward the R state that little oxygen would be released in physiological conditions. Thus, an additional mechanism is needed to

properly stabilize the T state.

This mechanism was discovered by comparing the oxygen-binding properties of hemoglobin in red blood cells with fully purified hemoglobin. Pure hemoglobin binds oxygen much more tightly than does hemoglobin in red blood cells. This dramatic difference is due to the presence within these cells of BPG. This highly anionic compound is present in red blood cells at approximately the same concentration as that of hemoglobin (~2 mM). Without 2,3-BPG, hemoglobin would be an extremely inefficient oxygen transporter, releasing only 8% of its cargo in the tissues.

How does 2,3-BPG lower the oxygen affinity of hemoglobin so significantly?

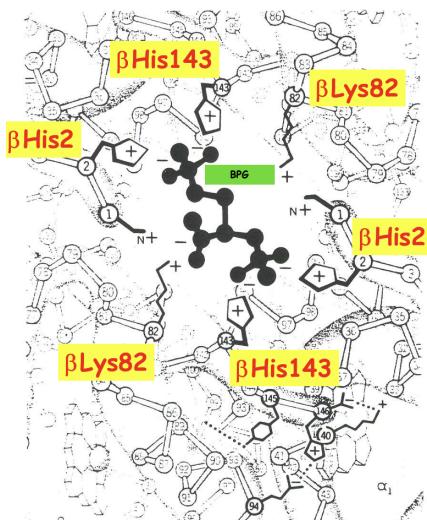
Examination of the crystal structure of deoxyhemoglobin in the presence of 2,3-BPG reveals that a single molecule of 2,3-BPG binds in the center of the tetramer, in a pocket present only in the T form.

On T-to-R transition, this pocket collapses and 2,3-BPG is released. Thus, in order for the structural transition from T to R to take place, the bonds between hemoglobin and 2,3-BPG must be broken.

In the presence of 2,3-BPG, more oxygen-binding sites within the hemoglobin tetramer must be occupied in order to induce the T-to-R transition, and so hemoglobin remains in the lower-affinity T state until higher oxygen concentrations are reached.

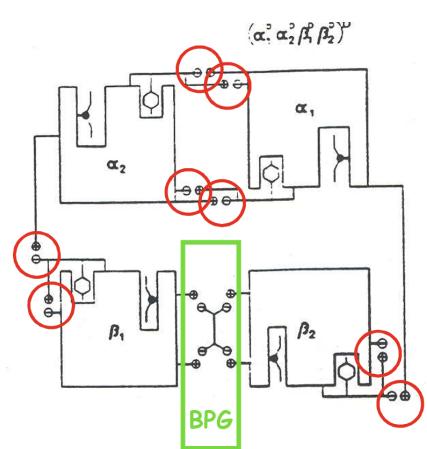
- BPG binds between the two beta chains
- The  $\alpha_1\beta_2$  and  $\alpha_2\beta_1$  dimers move relative to each other by a 15 degree rotation between T and R state

Amino acid side chains around the BPG site



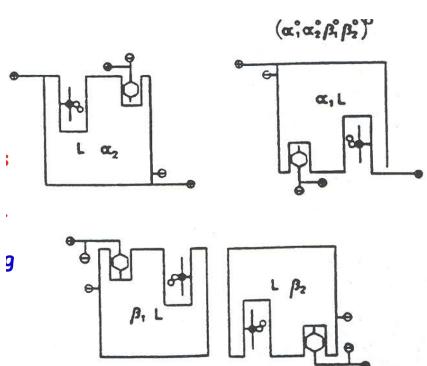
- This top view of the central cavity in human deoxy-Hb shows the positive charges lining the BPG site.
  - Six positive charges associate with 5 negative charges on BPG in the middle
- Fetal Hb loses two positive charges by replacing His143 with Ser143, decreasing the affinity for BPG, and increasing the O<sub>2</sub> affinity.
  - This difference in oxygen affinity allows oxygen to be effectively transferred from maternal to fetal red blood cells.

## The key - 8 salt bridges



T state - all four subunits have no O<sub>2</sub> bound at the haem

- Diagrammatic sketch of closing and formation of eight salt bridges by C-terminal residues in deoxy-Hb state
  - Note BPG is bound. This is allosteric effector and alters the T/R equilibrium towards the T state.



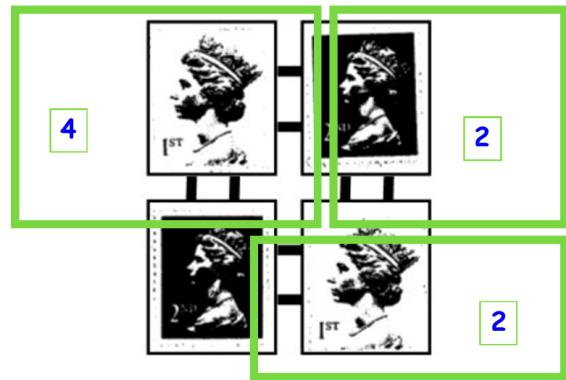
R state - all four subunits have O<sub>2</sub> bound at the haem

- The salt bridges must break to allow O<sub>2</sub> binding.
- Diagrammatic sketch of opening of salt bridges by C-terminal residues in oxy-Hb state.
- Note that heme group is now flat

- L=ligated

## Quaternary structure and allostericism - the postage stamp analogy

- Deoxy Hb is stabilised by 8 salt bridges that are broken when O<sub>2</sub> becomes bound
- The first O<sub>2</sub> molecule is bound with difficulty, but the 2nd, 3rd, 4th O<sub>2</sub> are bound more easily
  - Binding of first O<sub>2</sub> breaks 4 salt bridges
  - The second and third breaks 2
  - The fourth breaks 0 salt bridge, easily bound

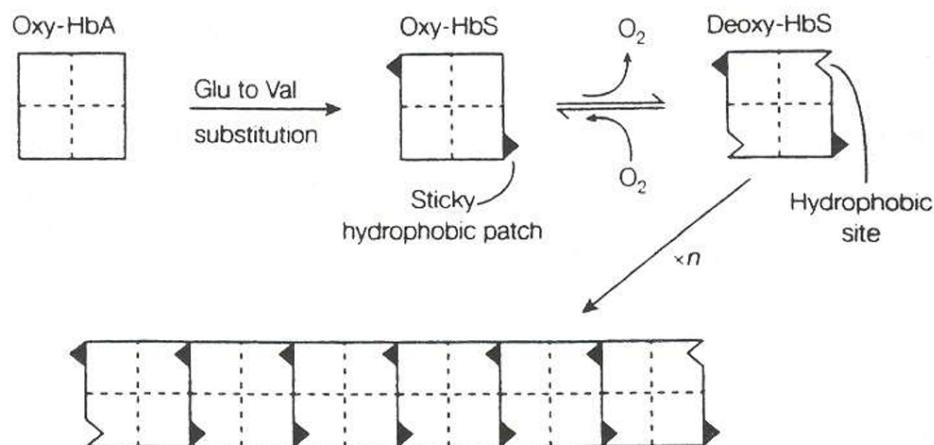


## Genetic diseases

### Sickle cell anemia

- Caused by an amino acid mutation in hemoglobin - betaGlu6 (hydrophilic) to betaVal6 (hydrophobic)
- Symptoms
  - Patients feel weak and dizzy with headaches
  - The heart can be enlarged
  - The blood is anemic
  - Examination of the RBC show "thin, elongated, sickle-shaped" appearances - caused by the fibres of Hb-S in these
- Sickling cycle:
  - Oxy Hb-S becomes deoxy Hb-S
    - Less sport
  - Precipitation of deoxy Hb-S as long fibres

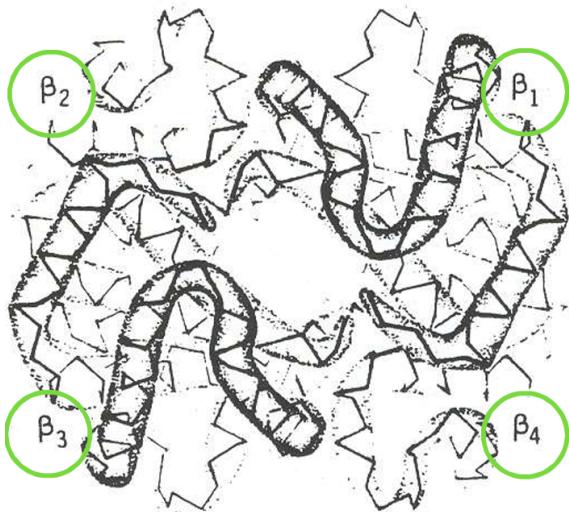
- Sickling of erythrocytes
- Blockage of blood vessel
- Local region of low O<sub>2</sub> tension
- More deoxygenation
- More sickling
- Causing Infarction
- Molecular basis for the aggregation of deoxyhemoglobin molecules



- A binding pocket is created in deoxy-Hb that can bind to the hydrophobic betaVal6 sidechain.
  - Oxy Hb-S will not cause sickling due to the absence of hydrophobic site
  - Many deoxy-Hb molecules associate to form a fibre

## Thalassaemia

- Caused by the loss or substantial reduction of a single hemoglobin chain.
  - The result is low levels of functional hemoglobin and a decreased production of red blood cells, which may lead to anemia, fatigue, pale skin, and spleen and liver malfunction
- Mediterranean populations are affected



- The symmetrical but non-functional hemoglobin H (beta4) is made up of four identical beta-chains in a disease called **alpha-thalassemia** in which no or very little α-chains are formed.
- These tetramers, referred to as hemoglobin H (HbH), bind oxygen with high affinity and no cooperativity.
  - Thus cannot behave like sigmoidal shape protein
  - Four identical b subunits come together with perfect 222 symmetry.
- Beta-thalassemia: b chain of hemoglobin is not produced in sufficient quantity.
  - In the absence of b chains, the a chains form insoluble aggregates that precipitate inside immature red blood cells. The loss of red blood cells results in anemia.