

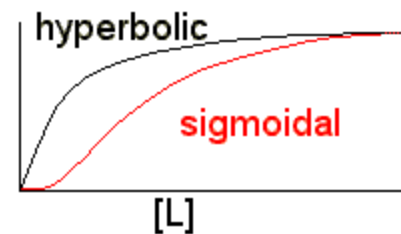
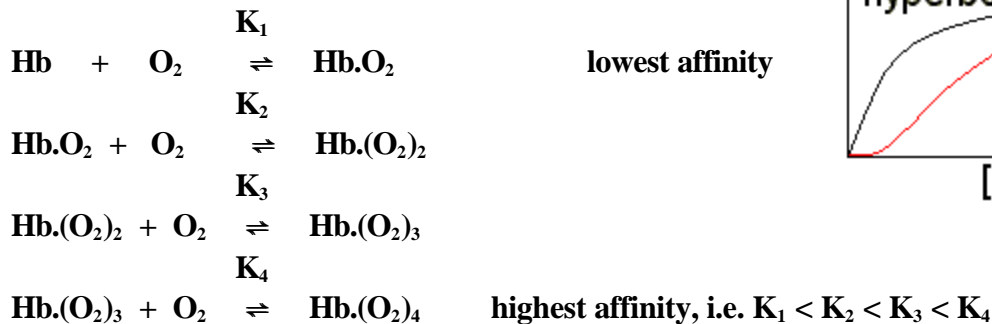
Chem*3560 Lecture 3: Cooperative behaviour of hemoglobin

Sigmoidal kinetics suggests that O₂ affinity increases with each O₂ bound by hemoglobin

Hill's theory requires that O₂ binding sites in hemoglobin should be occupied simultaneously, which is a bit too improbable, so a new theory is needed.

In 1925, Adair showed that the sigmoidal curve could be derived mathematically for O₂ binding in four consecutive steps, if it was assumed that **affinity for O₂ increased at each step**.

For each step there is an **affinity constant K**



This rules out sigmoidal behaviour for monomeric proteins like myoglobin.

However, if a protein contains **pre-existing** high and low affinity binding sites for the same ligand, the high affinity site should be occupied first, not last.

If the second site in hemoglobin has higher affinity than the first site occupied, this means that **affinity must be changing as each site is filled**.

If the hemoglobin conformation undergoes change due to induced fit as each site is occupied, this could bring about the observed change in binding affinity. However, changing the affinity of the site already occupied is not sufficient. What is necessary is for some mechanism that allows **occupancy of site 1 to affect the affinity of unoccupied site 2**. This is only possible if there are **multiple binding sites in one molecule**, as in hemoglobin, and not if the molecule only contains one binding site, as in myoglobin.

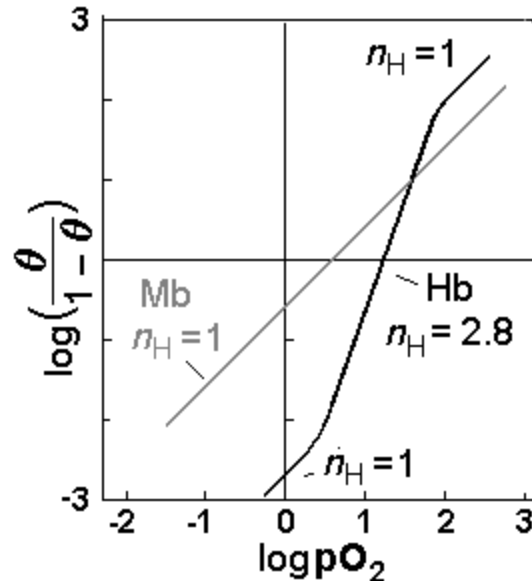
Cooperative effects imply that conformational changes can be induced both by the presence of bound ligand **and by the state of the immediate neighbours in the oligomer**. This allows **unoccupied neighbours of an occupied subunit to switch to the high affinity state**.

The allosteric model of cooperative behaviour

The **Hill coefficient** n_H , the slope of the line in the Hill plot, is therefore given a new meaning to replace the original concept of simultaneous binding in multiple sites. This new definition allows for non-integer values, and values less than 1.

A protein such as hemoglobin shows **positive cooperativity** when apparent affinity increases with increasing occupancy ($n_H > 1$).

Some proteins show **negative cooperativity** where affinity decreases with increasing occupancy, ($n_H < 1$).



Structural studies of deoxyhemoglobin (fully vacant) and oxyhemoglobin (fully occupied) **show two distinct conformations** (Lehninger pp. 212-213).

T-state (*tense*) has lower O_2 affinity and exists at low occupancy.

R-state (*relaxed*) has higher O_2 affinity and exists at high occupancy.

Regulation based on the switch between two structures is called **allosteric control** (*allosteric* is Greek for *other shape*).

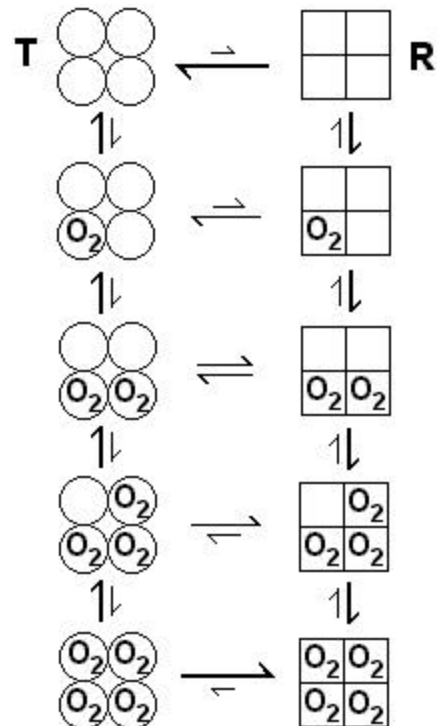
Allosteric control can affect an enzyme's affinity for substrates or its rate of catalysis. Proteins subject to allosteric control use one of two mechanisms, the **concerted** model or the **sequential** model.

The concerted model for cooperative behaviour

(Lehninger p. 216)

The **concerted** or **MWC model** (for **M**onod, **W**yman and **C**hangeux) assumes that O_2 molecules bind to Hb **one by one** until all four sites are occupied. Increasing occupancy increases the probability that a hemoglobin molecule will switch from T to R state.

The **key concept** of the MWC model is that **all subunits** in one Hb molecule switch state **simultaneously**, due to **symmetry relationships** between the subunits. This allows unoccupied subunits to adopt the high affinity R-state.



Note that the equilibria in the T to R transitions and O₂ binding steps are all **biased** on one direction or the other. Fully vacant Hb strongly favours T-state.

When Hb binds the first O₂, induced fit favours a switch to R-state, but the presence of three T-state neighbours holds back the changeover. Therefore Hb.O₂ tends to stay in the T-state.

The probability of switching to R-state increases when the second O₂ binds.

By the time the third O₂ binds, the switch to R-state is likely to have occurred; the three occupied R-subunits have forced the unoccupied subunit to switch, so the final O₂ binds with high affinity.

Because of biased directions of the various equilibria, T-state molecules tend to release O₂ and become empty while R-state molecules tend to fill up. A sample with average occupancy $\theta = 0.5$ will be composed of a mixture of Hb and Hb.(O₂)₄, with very little Hb(O₂)₂. A tetramer that lacked cooperative behaviour would be mostly PL₂ at the $\theta = 0.5$ occupancy level.

The sequential model is an alternative

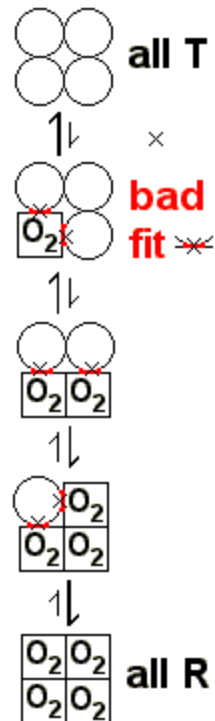
The **sequential** or **KNF model** (for **K**oshland, **N**emethy and **F**ilmer) was devised because of doubts that all subunits should switch state concurrently, as required in the MWC model. (Lehninger fig. 7-14 b is more complicated than it need be for the KNF model).

The essence of KNF is that subunits **switch T to R only when occupied**, by direct induced fit action. Likewise, they will switch back R to T when vacated. However, **T subunits do not fit well with R** at points of contact.

At step 1, **two bad areas of contact are created** between T and R subunits, and this consumes some of the binding energy so that affinity is lowered.

Differences between step 2 and step 3 are more subtle and reflect creation of more favourable R to R contacts.

At step 4, **two bad areas of contact are eliminated** as all four subunits become R. This adds to the binding energy, so that the final binding step has high affinity.



Both allosteric models predict the same binding curve

Both MWC and KNF predict a sigmoidal binding curve that matches the experimental data well. Both models yield apparent values for K₁, K₂, K₃ and K₄ that **increase progressively** as suggested by Adair. Adair provided a mathematical derivation of the sigmoid curve, but no mechanism; MWC and KNF give us plausible mechanisms.

Hence the mechanism can't be determined by kinetics alone and structural studies provide the supporting evidence.

Some proteins, including hemoglobin, fit the MWC model well

Other proteins seem to follow the KNF mechanism (in particular, KNF works well for negative cooperativity).

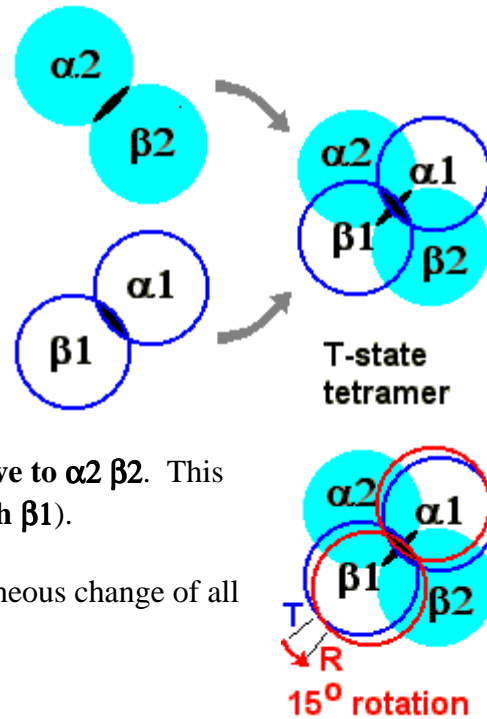
A closer look at hemoglobin structure

Hemoglobin consists of **two α -globin** subunits and **two β -globins**, making an asymmetrical tetrahedron, $\alpha_2\beta_2$.

The subunits are arranged as **two pairs, $\alpha_1\beta_1$ and $\alpha_2\beta_2$** . Little change occurs in the α_1 to β_1 and α_2 to β_2 interface, which is stronger than other contacts between subunits.

In the T to R transition, the $\alpha_1\beta_1$ pair rotates 15° relative to $\alpha_2\beta_2$. This **changes the way that α_1 interacts with β_2** (and α_2 with β_1).

Thus a **single global change** can account for the simultaneous change of all subunits between T and R state.



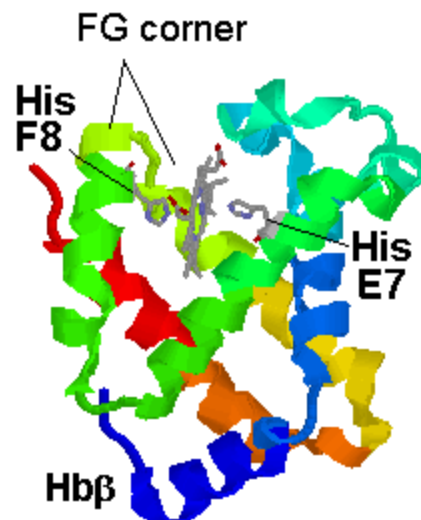
Changes during the T to R transition concentrate at the FG corner

Lehninger p.212-213.

The FG corner is the short connecting loop that links helix F to helix G.

Helix F	FG corner	Helix G
-His-Ala-Asp-Lys-Leu-Arg-Val-Asp-		
F8 F9 FG1 FG2 FG3 FG4 FG5 G1		

Key amino acids are bolded: note that **His F8 is ligand 5 for the heme- Fe^{2+}** .



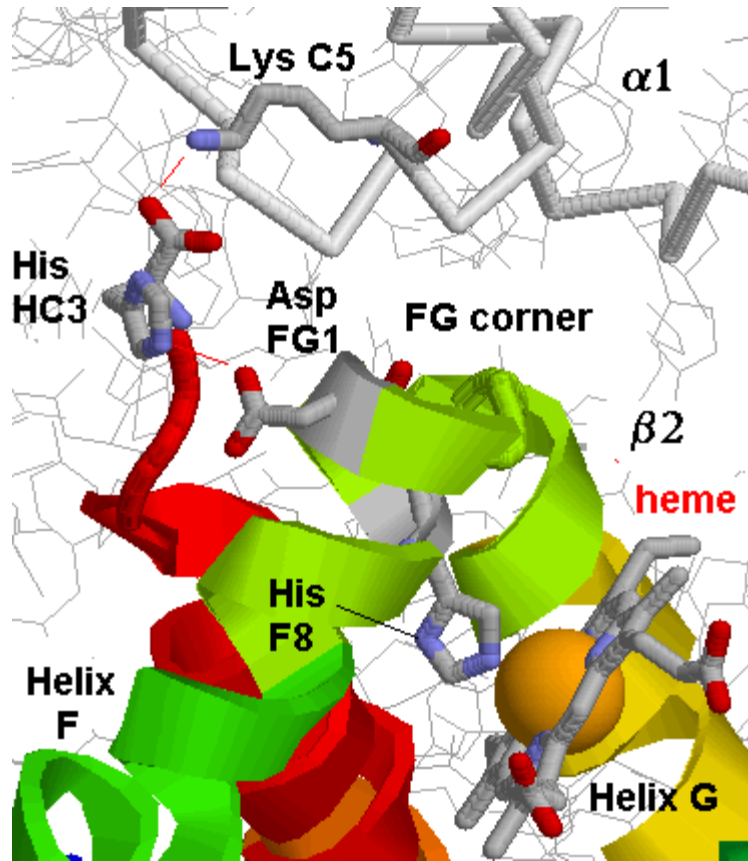
His HC3 is the C-terminal amino acid of the β -globin, and is positioned very close to the FG corner.

His HC3 forms two ion pairs in the T-state:

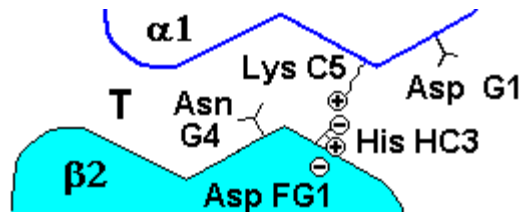
its C-terminal carboxylate group ion-pairs to **Lys C5** of the **opposite α -globin**, $\beta 2$ to $\alpha 1$ or $\beta 1$ to $\alpha 2$

its side chain ion pairs to the **Asp FG1** of the same β -globin.

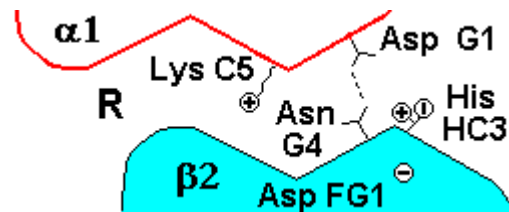
In the diagram, **O atoms are red**, **N atoms are blue** and **C atoms are grey** where individual amino acids are shown. The big rust-coloured atom in the middle of the heme is the **Fe²⁺**.



The schematic view on the right shows the region of contact between $\alpha 1$ and $\beta 2$, as aligned in the T-state above, and as aligned in the R-state below. **The $\alpha 1\beta 1$ globin pair rotates 15° relative to $\alpha 2\beta 2$** , so the $\alpha 1$ surface appears to slide over the $\beta 2$ surface

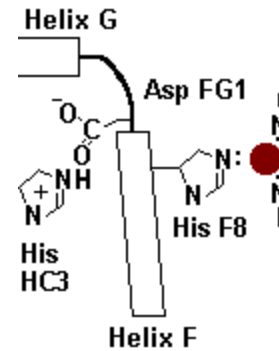


When the globin pairs rotate from the T-state position into the R-state position, the α -globin Lys C5 and the β -globin C-terminus **move apart**, and this causes the His HC3 to relocate and lose contact with Asp FG1.

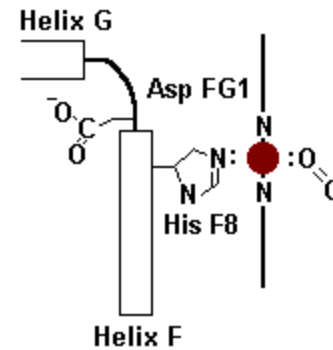


When the globin subunits align in the R-state, a new H-bond can form between the α -globin Asp G1 and the β -globin Asn G4, and this contributes to the stability of the R-state.

The ion pair between **His HC3** and **Asp FG1** that exists in the T-state **causes the F helix to tilt away from the heme**. Other amino acids hold the heme in position, so the heme becomes slightly puckered, but even so, **the Fe atom is pulled out of the plane of the heme towards its ligand 5, His F8**. This makes the heme Fe **less accessible to ligand 6 so that affinity for O₂ becomes much weaker** in the T-state of hemoglobin.



In the R-state, His HC3 is not in position to ion pair to Asp FG1, and **Helix F relaxes back towards the heme**. This allows the Fe atom to be **centered in the heme plane**, so that it is **fully accessible to ligand 6, and affinity for O₂ is high**.



When O₂ does succeed in binding to T-state deoxyhemoglobin, which it can do in the relatively high pO₂ found in the lungs, the ligand 6 O₂ **tends to pull Fe back into the plane of the heme**. This in turn exerts tension of His F8, which help the hemoglobin to switch into R state. The presence of a single bound O₂ in the hemoglobin tetramer may not be quite sufficient, but when two or three O₂ molecules bind, the probability of switching to R-state increases proportionately.

Likewise, when O₂ is released from oxyhemoglobin leaving ligand 6 vacant, the electrostatic attraction of His HC3 exerts force on Helix F to make it lean away from the heme. If two or three sites are vacant, the cumulative force may be sufficient to allow the switch back to the T-state.

When O₂ binds, it promotes the T → R switch
 T → R switch promotes more O₂ binding
 This happens best at higher pO₂, as occurs in the lungs

When O₂ is released, it promotes the R → T switch
 R → T switch promotes more O₂ release
 This happens best at lower pO₂, as occurs in the peripheral tissues.