

O₂ binding to haemoglobin

PDB 2HHB **PDB 1HHO**

This advanced worksheet requires the completion of the other haemoglobin worksheet first.

Load PDB 1HHO, which contains oxyhaemoglobin, and PDB 2HHB (in another tab), which contains deoxyhaemoglobin. Although PDB 1HHO only contains two chains, oxyhaemoglobin is made up of the same four chains as deoxyhaemoglobin *in vivo*.

The binding of molecular oxygen (O₂) to haemoglobin results in changes in the structure of the protein, termed **conformational changes**. Conformational changes are key to the function of haemoglobin and determine its affinity for O₂ (i.e. how easily O₂ binds). Here, we will attempt to identify how those changes occur.

Compare the secondary structure of oxy- and deoxy-haemoglobin by answering the questions below:

Does the secondary structure of the protein change upon O₂ binding?

The secondary structure of the protein remains the same upon O₂ binding.

Display the side chains of **His-58** and **His-87**. Those two residues are important for the function of the protein. One of them is called *proximal* (i.e. nearby) histidine while the other is called *distal* (i.e. distant) histidine, relative to the haem group.

Which residue is the proximal histidine and which is the distal one?

His-58 is the distal histidine while His-87 is the proximal histidine (bound to the haem) in chain A.

Examine the position of the O₂ molecule.

Which part of the haem binds O₂?

The iron binds molecular oxygen (O₂).

The distal histidine is known to bind and stabilise O₂.

What type of interaction may be responsible?

Electrostatic interactions hold the oxygen bound to the iron.

Examine the haem in oxy- (1HHO) and deoxyhaemoglobin (2HHB), as shown in Figure 3. One of them is truly planar (i.e. it lies completely flat) while the other is not (you may want to display **His-58** and **His-87** in deoxyhaemoglobin to make the comparison easier).

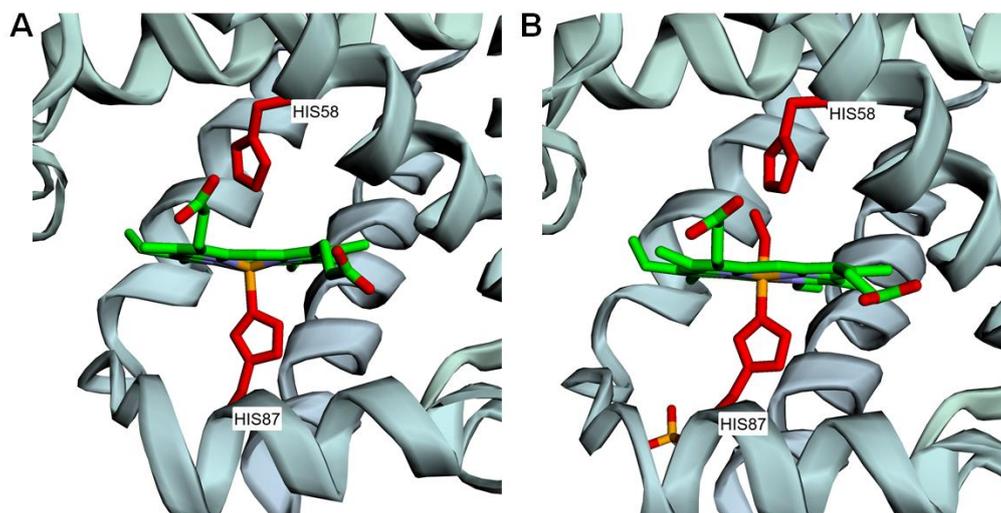


Figure 3 | Binding of O₂ to haemoglobin. **A.** O₂-free haem with His-58 and His-87 highlighted (PDB 2HHB). **B.** O₂-bound haem with His-58 and His-87 highlighted (PDB 1HHO).

Which haem group is truly planar (free haem or O₂-bound haem)?

The haem is truly planar when bound to O₂, in oxyhaemoglobin (PDB 1HHO).

Which atom lies out of the plane in the non-planar haem?

In deoxyhaemoglobin (PDB 2HHB), the iron lies slightly out of the plane that would otherwise be formed by the haem.

Bonus question: explain why a difference in planarity is observed.

Hint: This will require some knowledge of chemistry. Binding of O₂ to the iron can be thought of as an oxidation of the iron. What happens to the number of electrons around the iron nucleus? How can this be related to the size of the iron?

O₂ binding to the iron can be described as an oxidation of the iron. Oxidation can be defined as the loss of electrons; therefore the iron has fewer electrons in its electronic cloud when it is bound to O₂. This loss of electrons means that the iron is smaller (it has a smaller radius), allowing it to fit inside the haem. When it is not bound to O₂, however, the iron is larger and cannot fit inside the plane of the haem.

When the planarity of the haem changes, what happens to the proximal histidine?

As the proximal histidine is bound to the iron, it is pulled when the iron moves into the plane of the haem (i.e. upon O₂ binding).

How can that explain large conformational changes throughout the protein?

The proximal histidine is pulled by the iron, but its backbone is covalently attached to the rest of the polypeptide chain, so the movement of the histidine triggers large-scale movement of the protein.

Generate a figure to show how haemoglobin binds O₂. Include at least a view of the O₂-bound haem with the two functionally relevant histidine residues (with labels), like in Figure 3.