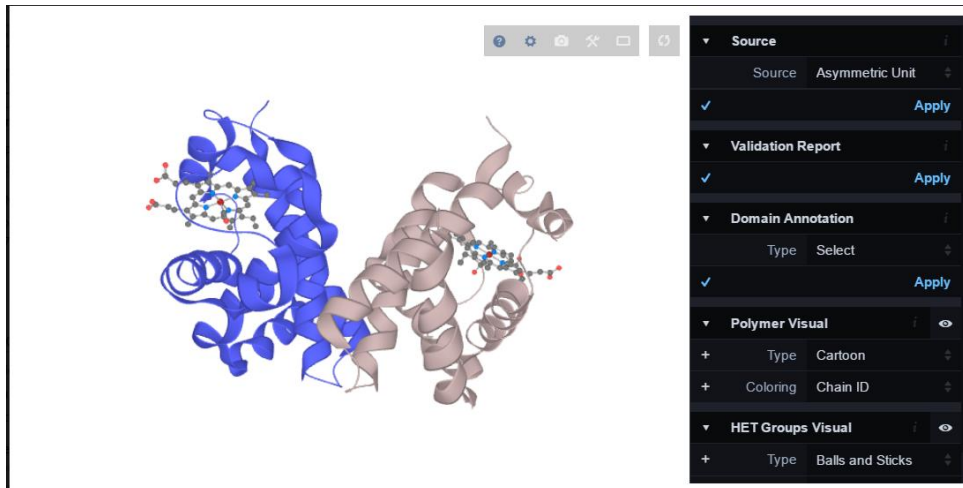


Name:  
Date:

# WA3 - Conjugated Proteins

Using the PDBe database (<http://www.ebi.ac.uk/pdbe/>) search for the entry: **2hco**, and load the 3D Visualisation. It should be a model of human carboxyhemoglobin which looks like this:



1. Which type of secondary structure can you see in this protein?  
 **$\alpha$ -helices**
2. The protein chains are connected to a porphyrin group which, in this case, carry carbon monoxide bonded to iron. What is the name of this type of structure?  
A **conjugated protein**. The porphyrin ring is called a prosthetic group.
3. Using the mouse, change the view around the protein so that you get a clear image of the porphyrin ring. Just using the automatic cartoon view, why do you think that the porphyrin ring binds to the polypeptide in that specific location?  
This part of the polypeptide is relatively open. It appears that the porphyrin group can slot in easily, and it has a **roughly complementary shape**.
4. Now change the Polymer Visual to 'VDW Balls'. Describe how the porphyrin group sits in relation to the overall shape of the molecule.  
It should be much clearer how the prosthetic group sits in the polypeptide. The VDW view shows a better representation of the actual size of the atoms in the protein. The porphyrin group sits almost perfectly in the structure, and is **highly complementary**. You could say that the site was designed or engineered for the prosthetic group.
5. Change the Polymer Visual again to 'Surface', how does this differ to the VDW Balls view? Why may there be some overlap with the porphyrin ring, and how would a CO molecule enter/leave the Fe binding site on porphyrin given that there appears to be a lot of protein in the way?

It appears that the porphyrin group is trapped in the polypeptide, and there is **a lot of overlap** between the two. This is partly because the structures take an average of the

positions of each atom based on its electron density. **The methods used are not 100% perfect, and although they are very accurate, there are always some small discrepancies.** As well as this, there is usually **some flexibility** in the molecule to allow for a good fit.

Small molecules such as CO, O<sub>2</sub> and NO can bind to the Fe ion in the centre of the porphyrin ring. There is some flexibility in the structure which allows the molecules to enter/leave and bind. It may be possible that there is an **induced fit mechanism**. Where once the small molecule binds, it creates a conformational change in the polypeptide chain around the prosthetic group and it appears to envelop it. However, we cannot know for certain, and there has to be some degree of uncertainty depending on the accuracy of the model. Although these models tend to be very accurate, because the atoms are so small it is never going to be 100% perfect!

6. Change the Polymer Visual back to 'Cartoon', to see the porphyrin ring more clearly, and click on the central atom, Fe, to bring it into focus. You should see all the neighbouring amino acid side chains come into view. Fe often exists as an octahedral complex, bound to six other atoms, but it appears that it is only bound to five, the one CO above it, and four from the porphyrin ring around it. By manipulating this view, deduce how the porphyrin group stays stable in its place. If it helps, change the magnification with the 'FOV' option accessible on the top right-hand side of the screen.

The Fe ion is arranged in an **octahedral** complex. It has four bonds to the porphyrin ring, one to the CO, and **one to the histidine residue below, via a N atom**.

7. Using your knowledge of proteins, predict what would happen if some of the amino acids in the polypeptide chain were changed.

Changing some of the amino acids would **alter the primary structure** of the polypeptide chain. This means that the polypeptide formed would end up **folding differently**, and produce a **different secondary, and therefore tertiary structure**. Changing amino acids forces the polypeptide to bind differently to accommodate the change, so the **active site would be different**, and this may stop the porphyrin ring from binding. Equally, there is still a possibility that changing the amino acids would produce a slightly better active site, and may even increase the binding affinity!

8. Explain why hemoglobin wouldn't be able to carry a large molecule such as glucose on the iron.

A large molecule such as glucose would be **too large to fit in the active site**.