Name:
Date:

WC1 – Stereospecific Active Sites

Download and then open the file **1h9z_Warfarin_Enantiomers** using PyMOL. This is a model of a human serum albumin protein. This is a protein which travels in the blood plasma and transports drugs around the body through the bloodstream. Attached to the protein are the two enantiomers of the drug warfarin, known as S-warfarin and R-warfarin, which are mirror images of each other.
Warfarin is an anti-coagulant drug that preventing blood clots, which if left untreated, can result in deep vein thrombosis, leading to possible strokes and heart attacks (myocardial infarctions).
Warfarin is sold as a racemic mixture – a 50:50 mixture of each enantiomer.
One of the enantiomers is more potent, and induces a stronger reaction than the other.

1. Using the command **S-Warfarin>A>orient**, focus in on the molecule. By holding the right mouse button and moving the mouse, zoom in/out to get a clear view of the drug. You can move the position of the camera by holding and panning the left mouse button. Use the command **S-Warfarin>S>as>spheres**, to show a 3D view of S-warfarin. Describe the relationship between the active site and S-warfarin.
   The active site has a **complementary** relationship to the drug. This means that S-warfarin fits well within the active site. There isn't much space left, and it is a tight fit.

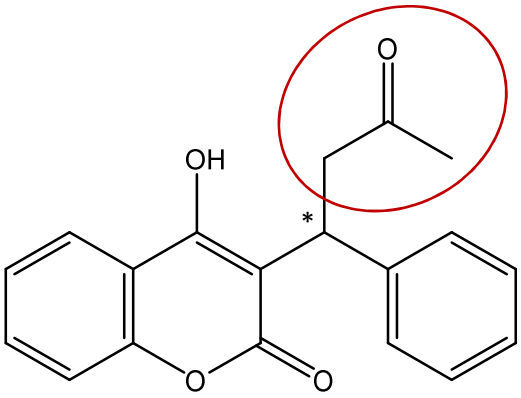
2. Deselect S-Warfarin and then select R-Warfarin. Using the command **R-Warfarin>S>as>spheres**, to show a 3D view of R-warfarin. Describe the relationship between the active site and R-warfarin.
   Almost exactly the same as with S-warfarin. The active site has a **complementary** relationship to the drug. This means that S-warfarin fits well within the active site. There isn't much space left, and it is a tight fit.

3. Now reselect S-Warfarin. Compare the two enantiomers in the active site. Does any one appear to be a better fit than the other?
   Not significantly, at least it doesn't appear that there is much of a difference between them, even though they are slightly different.

4. We know that one of the enantiomers is more potent – it has a stronger effect – than the other enantiomer. If the two enantiomers appear to have a complementary shape to the active site, what else may cause the two enantiomers to have different effects in the body?
   One enantiomer may be broken down faster than the other, so doesn't reach the target protein.
   The two drugs may **interact differently with another protein in the body** – remember that this protein just transports warfarin around the body, it isn't the final target protein.
   The drugs may have a complementary shape, but **may not have complementary interactions** – one may interact more favourably than the other.

Produced by Adam Stubbs at Newcastle University as part of a summer outreach project.

5. Using the command chain **S > as > sticks**, show both of the enantiomers. This will show both of the enantiomers partially superimposed. On the diagram below, circle the part of the molecule which is arranged differently in each enantiomer. Also, label the chiral centre.



6. Now by selecting **S-Warfarin_polar** and **R-Warfarin_polar**, show the polar interactions between each enantiomer and the protein. Which enantiomer interacts the most with the protein?
R-warfarin

7. Use the command **1h9z_Human_Albumin>S>as>sticks**. This will show the amino acid structures around the active site. Two of these residues will be interacting directly with warfarin. Select both of the residues, which should be highlighted as 222R and 242H in the residue bar at the top. Using the command **(sele)>C>by element**, explain why one of the enantiomers can interact more with the active site than the other.
R-warfarin can interact with the nitrogenous residues more than S-warfarin can. This is because **S-warfarin has the wrong geometry** in the active site, and the carbonyl group **cannot hydrogen bond** to the amino acids in the active site. Even though this is a small structural change, it means that one enantiomer can bind strongly, and the other, not as strongly. This allows the protein's active site to differentiate between the two enantiomers.

8. S-warfarin is a more potent drug than R-warfarin. Using your previous answers regarding the strength of the interactions between each enantiomer and the transporter protein, predict why S-warfarin is has a greater biological effect than R-warfarin.
R-warfarin binds to the transporter protein more strongly than S-warfarin does. Therefore, less **R-warfarin may be released by the transporter protein** when/where it is needed. In this case, it is beneficial that S-warfarin can bind to the albumin protein, and yet doesn't bind too strongly. Drug-receptor interactions must be well balanced – if they don't interact strongly enough then the drug won't be able to induce an effect. If it interacts too strongly, then it may block the active site, or trigger too great an effect, having other consequences.