WA6 –Ibuprofen as an Inhibitor

Download and open the file **1igz_1eqg_Ibuprofen_COX_Animation** using PyMOL. This is a model of the enzyme prostaglandin synthase, which is also known as COX-2. This is an enzyme which converts fatty acids such as linoleic acid into prostaglandins. Prostaglandins are the chemicals which trigger pain and inflammation in the body. In this exercise, we will explore how ibuprofen can be used to stop prostaglandins being produced, and therefore treat pain and inflammation.

1. What is the most common form of secondary structure in the protein COX-2?
   α-helices

2. Now, **select Linoleic_Acid** to view the substrate in the enzyme. Linoleic acid is a long chain carboxylic acid – a type of fatty acid. COX-2 is an enzyme which converts fatty acids into prostaglandins. Where is the substrate linoleic acid located on the enzyme? Is it buried deep within the protein, or is it bound on the surface?
   Deep **within** the core of the protein.

3. To explore the active site of the enzyme, select **1igz>S>as>Surface**. This will show the surface of the protein as it appears to a substrate. Now using the command **Linoleic_Acid>A>orient**, zoom in on the substrate. By clicking the left mouse button, you can change your view of the substrate. Describe how the substrate sits in the active site, what type of relationship do they have?
   The substrate sits in the active site well, **surrounded** by the surface of the protein. There is a **complementary relationship** between the active site and the substrate.

4. The active site is made from a series of amino acids, forming a pocket for the substrate to enter. Various studies have shown that amino acid #355 in the protein is crucial to the activity of COX enzymes. **Select Amino_Acid_355** to view the amino acid. Using the mouse to move the camera, and zoom in/out, observe the amino acid near the substrate. Predict what would happen if this amino acid was substituted with another one which was considerably larger. Also, predict what would happen if it was substituted for one which was considerably smaller.
   If it was substituted for a **larger amino acid**, then the **active site would be smaller**, and the substrate would **not fit** as easily in the active site. This would **reduce the complementarity** of the active site and the substrate.

   If it was substituted for a **smaller amino acid**, then the **active site would be larger**, and the substrate would **not fit as tightly** in the active site. This would **reduce the complementarity** of the active site and the substrate.

Produced by Adam Stubbs at Newcastle University as part of a summer outreach project.
Modified from an exercise produced by Paul Cook at Grand Valley State University.

5. Ibuprofen is a type of non-steroidal anti-inflammatory drug, NSAID. It is known to bind to the COX-2 enzyme. **Select Ibuprofen** to show how ibuprofen binds to the enzyme. Where does ibuprofen bind to the enzyme?
   Ibuprofen binds in the same **active site** as the linoleic acid substrate.

6. Using this information, explain how ibuprofen can stop the COX-2 enzyme from converting linoleic acid into the prostaglandins.
   Ibuprofen binds to the enzyme in the **active site**. This **stops linoleic acid from binding**, by working as an **inhibitor**.

7. Predict what would happen to the effectiveness of ibuprofen if the concentration of linoleic acid was greatly increased.
   Increasing the concentration of linoleic acid would mean that it would be **more likely that a molecule of linoleic acid binds to an active site than a molecule of ibuprofen does**. This would **reduce** the effect of the ibuprofen.

8. **Deselect 1igz and Amino_Acid_355** to show only the linoleic acid substrate, and the ibuprofen molecule. By comparing the two molecules, explain how you could design another drug to act as an inhibitor.
   The ibuprofen inhibitor has a **similar shape** to part of the substrate. To design an **inhibitor**, it should have a **similar structure to the substrate**, to increase the chance that it will bind **effectively to the active site**, by being **complementary**.

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