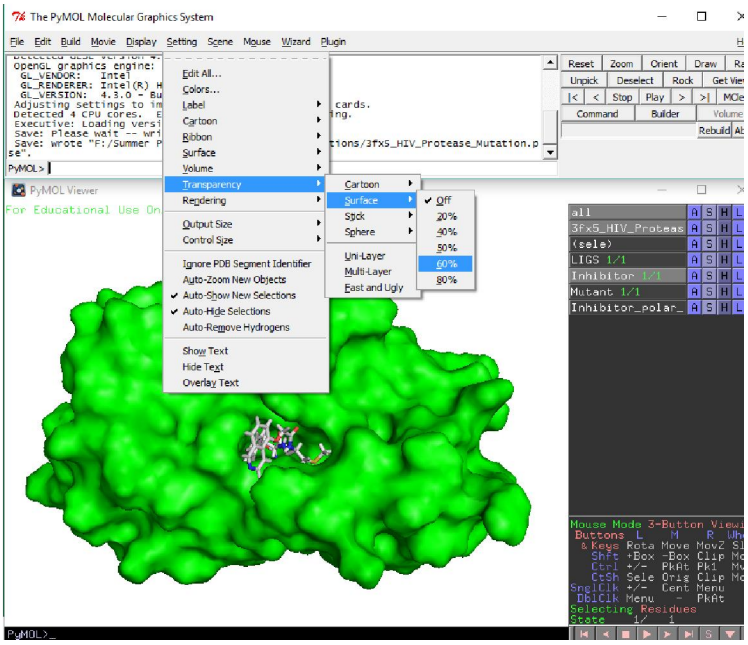
WA5- Active Sites and Mutations

Download and then open the file 3fx5_HIV_Protease in PyMOL. This is a HIV protease protein, bound to an inhibitor. HIV protease is one of the key proteins within the HIV virus. As a virus, HIV will reproduce inside the body, and this requires it to produce certain necessary proteins. The HIV protease enzyme is one of the essential proteins which helps HIV to replicate in the body, by assisting with protein replication. The purpose of an inhibitor is to stop this process from happening. If an inhibitor is successful in doing this, then it has the potential to be used as a drug to treat HIV/AIDS.

1. Protease will cut larger polypeptides into shorter, specialised ones. Using the command **HIV_Protease>S>as>surface**, show the surface of the protein structure. Using your knowledge of inhibitors, explain how the inhibitor shown stops the enzyme from working effectively.
   The inhibitor works by **blocking the active site** of the protease enzyme. The inhibitor itself appears to have a good fit to the active site, making it **complementary**. Because it blocks the active site, the enzyme is unable to work effectively, as the polypeptides which it is supposed to cleave cannot enter the blocked active site. If the enzyme cannot perform its function, then the HIV will struggle to replicate in the body. This inhibitor therefore has potential to treat HIV and reduce the effects of the virus on the body.



2. Set the transparency of the surface using the command **Setting>transparency>surface>60%** on the top toolbar.
   Now select the **Inhibitor_polar_conts_** to show the interactions between the inhibitor and the enzyme. How is the inhibitor held in the active site of the enzyme?
   The inhibitor is held in place by **polar interactions** with the enzyme, such as **hydrogen bonds**. These interactions are along the entire length of the inhibitor molecule, and hold the inhibitor, thus blocking the active site.

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

3. What would happen to the effectiveness of the inhibitor, and of the HIV protease enzyme, if there were more of these interactions in place?
   The **inhibitor would become more effective**, and the **enzyme would therefore become less effective**.

4. Why is it important that the interacting groups, the ones which bind to the enzyme, are in the specific locations that they are? What would happen if they were replaced, or changed position slightly?
   It is important because the **active site has a very specific structure**, which binds to the inhibitor. The active site is made from amino acids organised into a 3D structure. Each amino acid has **very specific interactions** with the inhibitor, and the overall tertiary structure of the active site is therefore very specific. If some of the interacting groups were changed in some way, then they may **not be able to interact as effectively** with the enzyme's active site, and the **inhibitor may be less effective**.

5. The inhibitor contains multiple chiral centres. Why is it important to consider these when designing a drug?
   Each chiral centre can be arranged in two different ways, giving two **different enantiomers**. The more chiral centres that a molecule has, the more enantiomers it can have. So, if a molecule has 2 chiral centres, then it can have 4 different enantiomers (RR, RS, SR, SS). Each **enantiomer will have a slightly different structure** and shape in space, and they **may not all bind effectively to the active site**. This is because the **active site is stereoselective**, and can distinguish between two different mirror image enantiomers. This means that one enantiomer may be very effective, but another may be less so, or may even cause other unknown side effects.

6. Using the command **Setting>Transparency>Surface>off**, remove the transparency of the enzyme. We will now consider the effect of mutations in a protein.
   Enzymes are proteins made from chains of amino acids. These amino acids are coded for by DNA. What could happen to the structure of the enzyme if the DNA code is changed slightly?
   This mutation could cause the **amino acids in the protein to change**, and therefore the **primary structure would be different**. This will have a knock-on effect to the secondary structure, and then the tertiary structure of the protein. A small change in the DNA code can easily **alter the shape and therefore function of the enzyme**.

7. In PyMOL, a mutated enzyme has been created. This has been done by duplicating the enzyme, but replacing just one of the amino acids in the sequence with a different one. In this case, a valine has been replaced with a tryptophan**. Select Mutant in the right-hand pane to overlay the mutated enzyme onto the healthy HIV protease and the inhibitor**. How does the mutated enzyme compare to the healthy one, and what impact could this have on the inhibitor?
   The mutated enzyme has replaced a small valine amino acid with a **large and bulky** tryptophan. This **partially blocks the active site**, and overlaps with the inhibitor. There is a

clash between the inhibitor and the new mutation, which could **prevent the inhibitor from binding**. This may make the inhibitor useless as a drug.

8. Mutations happen randomly in the DNA, and this mutation could have occurred anywhere in the amino acid sequence – the primary structure. The mutation is equally likely to occur anywhere else in the molecule. What effect would this have on the overall function of the enzyme, and the effectiveness of the inhibitor, if the mutation happened away from the active site?
   If the mutation happened away from the active site, then the **enzyme may appear to be unaffected**, and the **inhibitor may work as expected**, preventing HIV protease from working, and treating HIV. But there is still a **chance** that by binding away from the active site, it would change the way that the **polypeptide chain folds in 3D**. This means that it **may in fact still affect the tertiary structure of the enzyme**, and this should still be considered. There is a chance that the mutation may change the shape of the active site slightly, which may cause the inhibitor ligand to **bind stronger, making it more effective, or weaker, making it less effective**.

9. Why is it useful to use computational models such as PyMOL when designing drugs and inhibitors such as this?
   It allows the inhibitor and drugs to be **visualised in 3D**. This means that we can see the **drug docked into the active site**, and predict how the binding will change as the inhibitor changes. For example, we may notice that there is part of the inhibitor which isn't binding to the active site, and it may be possible to **change the design of the inhibitor**, to make it **bind more effectively**, such as by changing one of the group on the molecule.
   It also allows any changes such as **mutations to be computed**. It is much, much easier to use a computer model to see the effect of mutations on the active site and ligand binding, than it is to physically mutate a HIV protein in the laboratory and then experimentally determine the effects!

10. The inhibitor is shown to be a very potent inhibitor. What else should be considered before producing and using this drug on the mass market as a treatment to HIV?
    Even though the drug is very effective, it is important to consider **other safety aspects** too. Such as any **side-effects** that the drug may have, and other **drug-drug interactions** which may occur. It's also important to consider the **conditions that the research was done in**. Was this performed on human patients under normal conditions, or was it done on a lab rat, or in a petri dish? Then you must consider other effects such as **solubility** and **absorption** in the body – can the drug get to the desired target in the body without being digested? Finally, it is still important to look at the **cost of production**. Even if the drug is the most effective on the market, is it still feasible to use if it would cost 25x more to produce than the current treatment options? These factors will have to be considered at some point, and this is why it can take many years, and easily over a decade to produce a drug which is sold and used medicinally. This is without considering the various stages of **drug testing**, and the various **legal obligations**.