Structural Biology Exercise #3
Why are statins powerful inhibitors of cholesterol synthesis?"

GOALS OF THE PRACTICAL:
1. Get more familiar with proteopedia, protein data bank and pymol
2. How to find information about the structure
3. How to analyze protein-ligand interactions

1. What is the target protein for statins?

Go to Proteopedia (http://www.proteopedia.org) and search for "Lipitor". Lipitor is an inhibitor of cholesterol synthesis and the best selling drug in the world. On the page you find information how the drug works and affects the target. On the right you see a Jmol window displaying the structure of the drug. You can manipulate the view with mouse. While reading the text you can click on green links to update the view of the molecule. When you have read the page, follow the link with the name of the Lipitor target protein (HMG-CoA Reductase).

2. Learn more about the target protein

Read the text on the target protein page and click on the green links to visualize the molecule regarding the data on it. What is the biological function of the target protein? ("It catalyzes the formation of mevalonic acid, the committed step in the biosynthesis of sterols, most notably cholesterol.")

Under **Additional 3D Structures of HMG-CoA Reductase** follow the link with the PDB (protein data bank) code 1HWK. What is this structure? (Complex of the catalytic portion of human HGM-CoA reductase with Atorvastatin)

2. Search and save the coordinates from PDB.

Go to PDB web site http://www.pdb.org

Search for the PDB code with 1HWK

Note the information available on the summary page (primary citation, related pdb entries, ligands, ...) and in other tabs (sequence, annotations, ...)

Save the coordinates for the structure.

3. Search information about ligand interactions from PDBSum

Select the **Links** page and the **PDBSum** link on the page.
Under **Ligand**, select **117** to see how Atorvastatin binds its target protein.

Under **LIGPLOT of interactions involving ligand,** select the .pdf link to view the interaction more clearly. Save the .pdf file.

3. Analyze how Atorvastatin binds to HGM-CoA reductase.

Open Pymol and on upper **Command window** *File/open* and select the 1HWK.pdb file (Note: You have to first search for the directory where you saved this file).

Color carbon atoms in each of the chain of the tetramer with a different color:
From the upper window, select *Display/Sequence* and select *Display/Sequence mode/Chains* and select *A* from the sequence line. Color chain A: (*sele) C/by element* and select the option with cyan carbon atoms. Unselect everything by clicking black space on the PyMol Viewer window. Color the chain B with a magenta color (Use (*sele) C/by element* and select magenta color). Unselect everything. Color the chain C in the same way with salmon. Now the chains in the structure are displayed with different colors. Center the view *1HWK A/center*.

Save the scene by selecting from the pull-downs scene/store/F10.
You can return to this scene later by pressing F10 on the keyboard.

To hide everything select *1HWK /H/everything.*

Color the carbon atoms in Atorvastatin with blue (violet) color: From the upper window, *Display/Sequence mode/Residues.* Now you see all the residues, which are included in the pdb file and you can select residues from the sequence line.

To select the Atorvastatin ligand, select *117* on the sequence line.

Select *sele S/sticks* to see Atorvastatin and color Atorvastatin with blue (Use (*sele) C/by element* and select the color).

Show the residues that are from 4 Å distance from Atorvastatin: (*sele) A/modify/expand by 4Å, residues*. Then visualize these residues (*sele) S/sticks.* Center the view (*sele) A/center* and zoom the view (*sele) A/zoom.* (Unselect everything by clicking black space on the PyMol Viewer window). Rotate the view to analyze Atorvastatin binding site.

Where is the binding site for Atorvastatin located in the target protein? (It is located between the two monomers and residues from both of them contribute to Atorvastatin binding.)

Save the scene by selecting from the pull-downs scene/store/F11.
You can return to this scene later by pressing F11 on the keyboard.

Analyze the interactions. Look at the interaction map (.pdf file) you saved from the PDBSum page to find out the interactions. The hydrogen bonds are indicated with a green dashed line and the number tells how long the distance is.

Show the hydrogen bonds with pymol:
The hydroxyl oxygen of Ser661 in the A chain (Ser661(A)) makes a hydrogen bond with a fluorine in Atorvastatin.
Let's draw this bond: From the upper window, select *Wizard/Measurement* and pick the oxygen in Ser661, then pick the fluorine in Atorvastatin. Similarly, draw the other hydrogen bonds. Press *Done* to finish measurements.

Which amino acids make hydrophobic contacts with Atorvastatin? (Val683(A), Gly560(B)), Leu562(B), Ala751(A), Leu853(B), Ala856(B), Leu857(B).