# Protein Studies Using CAChe

Exercise 1   Building the Molecules of Interest, and Using the Protein Data Bank

In the CAChe workspace, click File / Open, and navigate to the C:\Program Files\Fujitsu\ CAChe\Fragment Library\Steroid folder and double click on the Steroid.csf file. A generic steroid structure will be displayed on the screen.

Modify the structure and build **testosterone** by using the select tool (Arrow, 1st down) to highlight atoms to be removed (use "delete"), and replace with the appropriate groups. <u>BE SURE to use correct atom hybridizations and maintain the correct stereochemistry</u>. DO NOT use the Beautify / Comprehensive command, as this will invert stereochemistry at the chiral carbons. Beautify / Valence will add H's if needed. Once the proper structure is obtained, save as testosterone.csf in an appropriate location. Optimize the geometry using MM2.

Testosterone   **Metribolone**
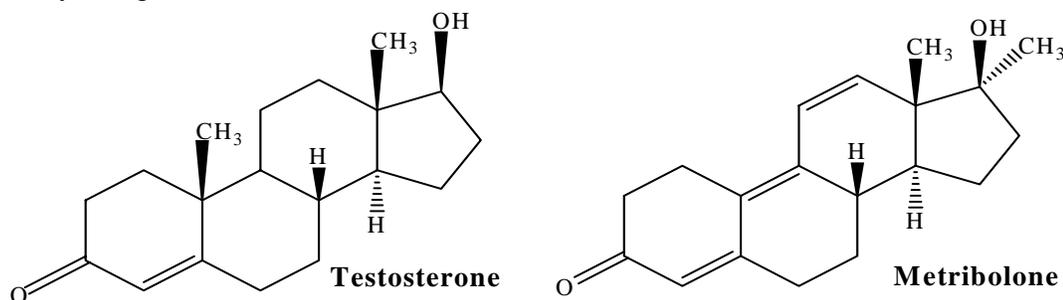
Open a new file, and build the Casodex molecule, save as casodex.csf, and optimize the geometry using MM2.

Casodex
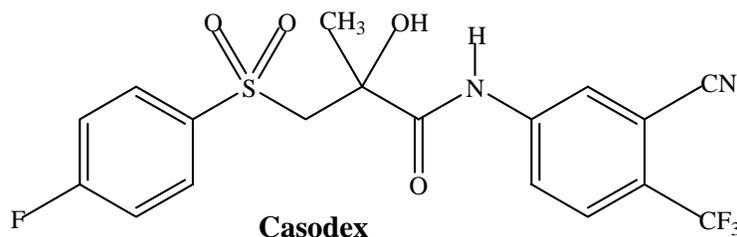
Open Internet Explorer and go to the Protein Data Bank web site at www.rcsb.org. Search for "human androgen receptor" – you should get three hits. The first of these is the one we are interested in. Click near the left side on the "1E3G" link to open a "Structure Explorer" window.

On the left margin, click "Download/Display File". Near the bottom of the new window that opens you should see "Download the Biological Unit File". Click on the link "1e3g.pdb1.gz" and save the file to an appropriate location on your computer (the Desktop is fine).

To "Unzip" this file, double click on it to open a two-pane window. In the right-hand window, RIGHT click on the "1e3g.pdb1" file, choose 'Extract to….", and pick a location on your computer (Desktop is good). **NOTE: The "unzip" protocol may be different on your computer.**

Rename the file (right click on the file, choose rename) to "1e3g.pdb" (i.e. – remove the "1" at the end of the name). You now have all the structure files you need to continue.

Exercise 2   The Protein/Metribolone Complex.

Experimental X-ray data of proteins contain water molecules, and also do NOT have all of the H atoms present in the structure since their positions are not determined experimentally.

In the CAChe Workspace, click File / Open and browse to the location of "1e3g.pdb". (NOTE: You may have to change "Files of Type" from .csf to "All files"). Double click "1e3g.pdb" to open the file.

We will first lock all the atoms in their crystallographic positions in space. Choose Edit / Select All to highlight all the atoms. Choose Adjust / Lock.

Choose View / Backbone Ribbon / Solid Ribbon. You can now see water molecules (red dots for oxygen) and the Metribolone ligand in the active site. Rotate the structure in order to view things better.

Choose View / Backbone Ribbon / Solid Ribbon to return to the "ball and stick" view. Using the Select Tool, click somewhere in the workspace away from the protein. The entire structure should now be "selected".

Choose Beautify / Valence. After a few seconds, white hydrogen atoms should appear. The H atoms can be hidden when needed by choosing "Suppress Hydrogens" under the View menu.

Choose Beautify / H-bonds. This rotates H atoms in –OH and water molecules to maximize hydrogen bonding. Choose Edit / Select All.

Choose File / Save As and use the name 1e3gclean.csf to save in an appropriate location. We will ignore the warnings about the charge.

Choose Adjust / Unlock, which will allow us to minimize the structure.

In order for the minimization to be finished in a reasonable amount of time, we will change the default values in CAChe. Read the following instructions carefully!

Choose File / Save As and use the name 1e3gmm2.csf to save the file.

Choose Edit / Select All .

Choose Experiment / New   Property of Chemical Sample, Optimized Geometry, using MM2, THEN click the "Edit" button in the Experiment window. This opens the CAChe Procedure Editor.

Double click on "Call MM-MM2_Geo using Sample1" to open another window. Click on "Edit" in the new window.

Double click on "Run Molecular Mechanics using Sample 1" to open the "Mechanics Settings" window. Near the bottom under Iteration Control, change the Maximum Updates: to 5000, and the Convergence Value: to 0.1, then click O.K. Close the edit windows you have open, and click "Yes" when asked if you want to "Save Changes to MM-MM2_Geo". When you are back to the original "Experiment" window, click "Start".

[NOTE: You have permanently changed the values used in this procedure. When finished with this lab, you can reset these values back to 300 updates and a convergence value of 0.001. This calculation illustrates the trade-off between computational cost and the amount of time you are willing to wait. Better results would be obtained using a lower convergence number, which you can experiment with on your own].

From the Experiment Status window, **<u>record the energy</u>** of the final structure here: _____

Close the Experimental Status and Experiment windows, but leave the protein display window open if you plan to proceed with the next step.

Exercise 3   Superimposing Testosterone on Metribolone in the Protein Active Site

The testosterone molecule will now be superimposed on Metribolone to see how well it will fit into the active site. This new structure will also have its geometry optimized.

If you had closed the file at the end of Exercise 2 above, re-open the 1e3gmm2.csf file. We will also open the testosterone file. Choose File / Open, and navigate to the location of testosterone.csf, and open this file in a new window. (Note that you now have two separate windows open – one may be directly underneath the other on the screen).

Click in the protein window to make this the active window. Choose View / Color by Molecule. The Metribolone should now be a different color than the protein. Use the rotate and zoom tools so that you can clearly see a few atoms of the Metribolone molecule.

Use the "Select Molecule" tool (2nd down, arrow with ring) and click on one of the atoms of the Metribolone molecule. It should be highlighted, while the protein is "grayed" out.

Choose View / Hide Unselected. You should now only see Metribolone.
Using a combination of the rotate tool and the zoom tool (magnifying glass), position the Metribolone molecule in an orientation similar to that shown on the next page. (NOTE: Even though the protein is not visible, it is also being rotated, etc.).

Click in the testosterone window to activate this window. Choose Edit / Select All, followed by Edit / Copy.

Click in the Metribolone window, and choose Edit / Paste. You will now see the testosterone molecule, probably on top of the Metribolone structure.

Choose Edit / Move Selected, and use the "hand" tool to move the testosterone molecule into a position adjacent to the Metribolone molecule. Use the rotate tool, the hand tool, the "Edit / Move Selected" choice, and the 'Select Molecule" tool to place the two molecules in a similar orientation adjacent to one another in the workspace. (See next page).

Next, we will prepare to superimpose testosterone onto Metribolone so that we can then delete Metribolone and have testosterone in the active site of the protein.

With only the testosterone molecule highlighted, choose Edit / Group Atoms. In the Group Type pull down menu, choose ligand, and type in "testosterone" for the Group Name. Click on the "Group" button. You should see testosterone appear in the defined groups window. Click on O.K.

Use the "Select Molecule" tool (2nd down, arrow with ring), and carefully click on one of the atoms of Metribolone. This molecule will now be highlighted. Choose View / Color by Element. Now choose Edit / Group Atoms. In the Group Type pull down menu, choose ligand, and type in "Metribolone" for the Group Name. Click on the "Group" button. You should see Metribolone appear in the defined groups window. Click on O.K.

Use the "Select Tool" (1st down, large arrow) and click on the carbonyl oxygen (**1**) of testosterone, **then holding the shift key down**, click on the atoms labeled **2**, **3**, and **4**. These four atoms should now be highlighted. SEE NEXT PAGE.

With the Select Tool, **hold the shift key down**, and click <u>in order</u> on the corresponding atoms in Metribolone (**5**, **6**, **7**, and **8**). These atoms should also be highlighted, as shown below:
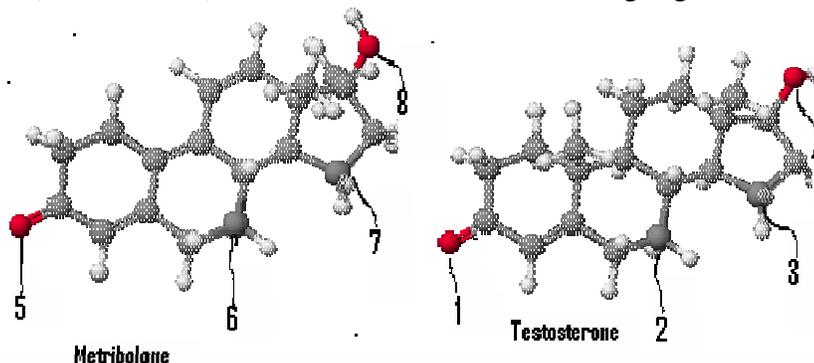
Choose Analyze / Superimpose. The testosterone molecule is now superimposed onto the Metribolone structure.
Choose View / Color by Molecule. Each of the molecules is now a different color.
Choose View / Show All. Now you can see the superimposed molecules in the active site.
Choose Edit / Select All, followed by File / Save As and use the name met+test.csf

Exercise 4   Replacing Metribolone with Testosterone

We can view the pocket in the protein structure that contains the bound ligand. This pocket is the active site of the protein. The surface of the protein that surrounds the ligand forms this active site.

Choose Edit / Group Atoms and click on testosterone in the "Defined Groups" list, and click on "G Only". This highlights testosterone and the rest of the structure is grayed out. Now click on Metribolone in the "Defined Groups" list, and choose "S or G". Click "O.K.". Now both ligands are highlighted, and everything else is grayed out.
Choose Analyze / Adjacent Surface – Pocket. Click "O.K.". After a few seconds you will see a surface drawn around the ligands.
Choose View / Hide Unselected. You now see the surface of the protein active site surrounding the two ligands. The surface has three colors that correspond to the following:

> <u>Blue</u>: An area where the protein donates hydrogen bonds. This reveals the location of hydrogen-donating AA residues on the protein.
> <u>Red</u>: An area where the protein accepts hydrogen bonds. This reveals the location of carbonyl =O atoms of the protein backbone.
> <u>Yellow</u>: An area of the protein that is hydrophobic.

Use the "Select Molecule" tool, and carefully click on the Metribolone molecule. A good place to do this would be on the methyl group attached to the same carbon as oxygen **8** shown above.
Choose Edit / Delete to remove the Metribolone molecule from the active site.
Choose Edit / Select All. Choose File / Save As and name the new file test+protein.csf.

Exercise 5   Evaluating and Refining the Docking of Testosterone with the Protein.

If is not already open, find the test+protein.csf file and open it.

Choose View / Show All, then choose View / Color by Molecule.

Choose Analyze / Label Hydrogen Bonds. This shows the distances for the three hydrogen bonds between the protein and testosterone (2 on the =O, and one on the OH of testosterone). Use the "zoom" tool if you have trouble finding these.

Choose Analyze / Label Bumps. For atoms on testosterone that are too close to protein atoms, distance labels appear. If you look closely (use the zoom), you can see most of these clustered near the methyl group on the "A" ring (the ring with the =O). This makes sense, because the Metribolone structure lacked this methyl group. The presence of these "bumps" reveals that the binding of testosterone may not be as good as that of Metribolone.

We can improve the fit by using molecular mechanics to allow both the ligand and protein to relax.

Choose Edit / Selcct All. Choose File / Save As and use the name test+proteinmm2.csf.

Choose Experiment / New   Property of: Chemical Sample, Optimized Geometry, using MM2, and click "Start".

[NOTE: In order for direct comparisons to make better sense, you need to make sure the settings used earlier (bottom of Page 2) are still in effect. When finished with this lab, you can reset these values back to 300 updates and a convergence value of 0.001, which are the default values for CAChe].

From the Experiment Status window, record the energy of the final structure here: _____

Compare this value with that obtained for the Metribolone/Protein complex on Page 2. Which ligand forms the more stable complex? _____   Does this make sense based on the known use for Metribolone? _____

Close the Experimental Status and Experiment windows, but leave the protein display window open if you plan to proceed with the next step.

Exercise 6   Superimposing Casodex on Testosterone in the Protein Active Site

Close the window containing the testosterone structure, if it is still open. If necessary, open the file test+proteinmm2.csf.

Choose File / Open, and navigate to the casodex.csf file you produced earlier.

Click in the protein window to make this the active window. Choose View / Color by Molecule. The testosterone should now be a different color than the protein. Use the rotate and zoom tools so that you can clearly see a few atoms of the testosterone molecule.

Use the "Select Molecule" tool (2nd down, arrow with ring) and click on one of the atoms of testosterone. It should now be highlighted, and the protein should be "grayed" out.

Choose View / Hide Unselected. You should now only see testosterone.

Using a combination of the rotate tool and the zoom tool (magnifying glass), position the testosterone molecule in an orientation similar to that shown below. (NOTE: Even though the protein is not visible, it is also being rotated, etc.).

Click in the Casodex window to activate this window. Choose Edit / Select All, followed by Edit / Copy.

Click in the protein window, and choose Edit / Paste. You will now see the Casodex molecule, probably on top of the testosterone structure.

Choose Edit / Move Selected, and use the "hand" tool to move the Casodex molecule into a position adjacent to the testosterone molecule. Use the rotate tool, the hand tool, the "Edit / Move Selected" choice, and the "Select Molecule" tool to place the two molecules in an orientation similar to that shown below. The fluorinated ring of Casodex and the "A" ring of testosterone (ring with =O) should be on the lower left.

Next, we will prepare to superimpose Casodex onto testosterone so that we can then delete testosterone and have Casodex in the active site of the protein.

With only the Casodex molecule highlighted, choose Edit / Group Atoms. In the Group Type pull down menu, choose ligand, and type in "Casodex" for the Group Name. Click on the "Group" button. You should see Casodex appear in the defined groups window. Click on O.K.

Note that testosterone already appears in the defined groups window. (If it does not, repeat the previous step for testosterone).

Use the "Select Tool" (1st down, large arrow) and click on the fluorine atom (**1**) of Casodex, **then holding the shift key down**, click on the atoms labeled **2**, **3**, and **4**. These four atoms should now be highlighted.

With the Select Tool, **hold the shift key down**, and click in order on the corresponding atoms in testosterone (**5**, **6**, **7**, and **8**). These atoms should also be highlighted, as shown below:
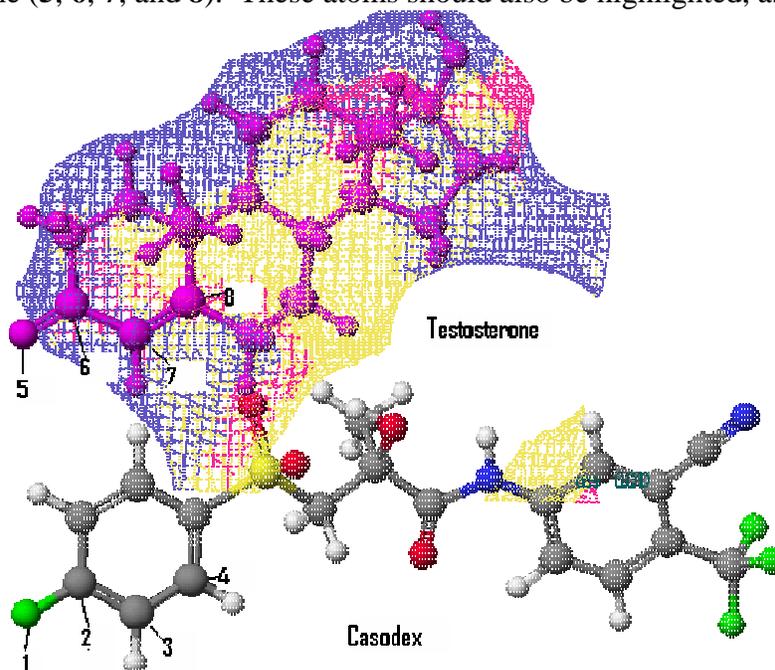
Choose Analyze / Superimpose. The Casodex molecule is now superimposed onto the testosterone structure. Since the structure is quite different, the fit is not good.
Choose View / Color by Molecule. Each of the molecules is now a different color.
Choose View / Show All. Now you can see the superimposed molecules in the active site.
Choose Edit / Select All, followed by File / Save As and use the name test+cas.csf.

Exercise 7   Replacing Testosterone with Casodex and Refining the Docking of Casodex with the Protein

Use the "Select Molecule" tool, and carefully click on the testosterone molecule.
Choose Edit / Delete to remove the testosterone molecule from the active site.
Choose Edit / Select All, followed by File / Save As and name the new file cas+protein.csf.
We can improve the fit by using molecular mechanics to allow both the ligand and protein to relax.
Choose Edit / Select All, then choose File / Save As using cas+proteinmm2.csf for the file name.
Choose Experiment / New   Property of: Chemical Sample, Optimized Geometry, using MM2, and click "Start".

[NOTE: In order for direct comparisons to make better sense, you need to make sure the settings used earlier (bottom of Page 2) are still in effect. When finished with this lab, you can reset these values back to 300 updates and a convergence value of 0.001, which are the default values for CAChe].

From the Experiment Status window, record the energy of the final structure here: _____

Compare this value with that obtained for the testosterone/Protein complex on Page 5. Which ligand forms the more stable complex? _____ Does this make sense based on the known use for Casodex? _____

Close the Experimental Status and Experiment windows.

We will now try a second orientation of Casodex that places the "other" substituted aromatic ring (with the cyano and trifluoromethyl groups) into the active site.

Repeat the above Exercise 6 and Exercise 7 steps, but now use the atoms shown on the next page to do the superposition:

Complete the Exercise 6 instructions, and save the file as test+cas2.csf.
Continue with the Exercise 7 directions, saving the file as cas2+proteinmm2.csf.
Run the calculation.
From the Experiment Status window, record the energy of the final structure here: _____
Compare this value with that obtained for original orientation of Casodex in the active site.
Which orientation is preferred? _____
Do the calculated values for both Casodex orientations make sense with respect to the energy calculated for the testosterone? _____

Close the Experimental Status and Experiment windows, but leave the protein display window
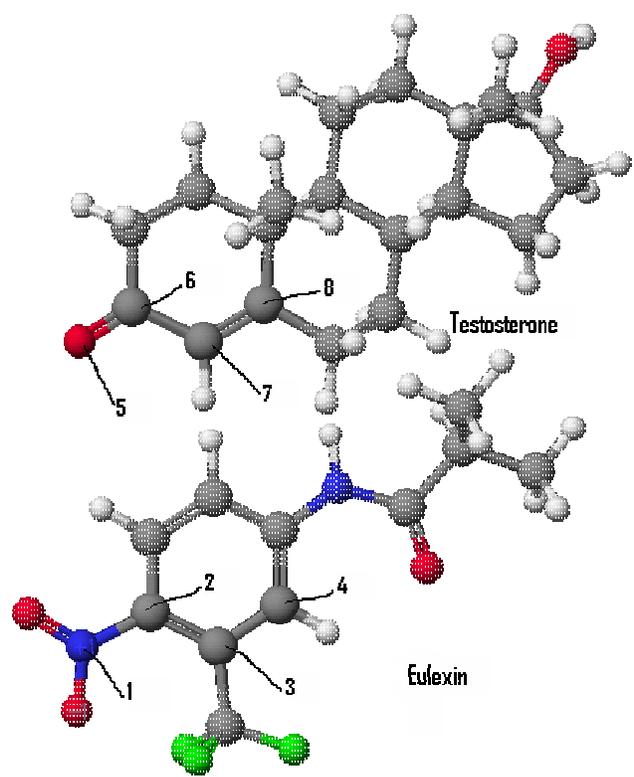open if you plan to proceed with the next step.
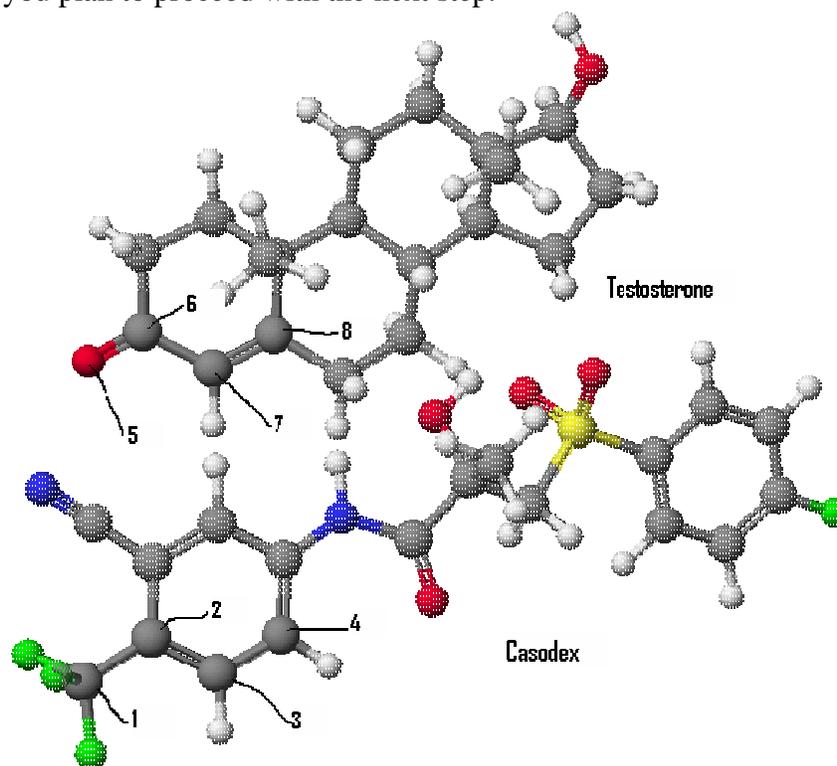
Exercise 8 **Investigating Eulexin as an Antiandrogen drug**

Another drug used is Eulexin, whose structure is shown below:
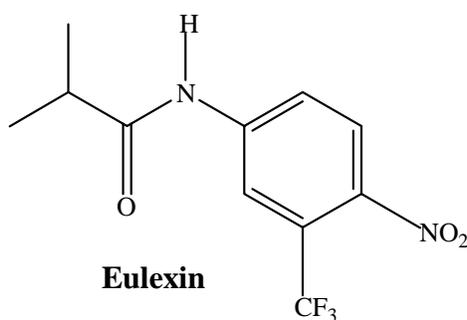
Eulexin

Repeat Exercises 6 and 7 but now superimpose Eulexin on testosterone. A suggested choice of
atoms for doing this is shown on the next page.

Once you have found the energy of the Eulexin/protein structure, record the value here_____.

Compare this energy with your previous results, and see if the relative energies make sense, based on the function of the various molecules.