

# Tutorial: Superimposing 3D Structures of Proteins with Swiss-Pdb Viewer

In order to be able to perform any kind of structural comparison between molecules, you need to first know how to use your tools well and also know how to visualize your molecules to obtain the correct information from superimposing two protein structures. Following the steps in this tutorial should give you a foundation for performing structural comparisons using Swiss-Pdb Viewer. You should be able to add more to your knowledge later from reading the Swiss-Pdb Viewer manual.

## Download and Install Swiss-Pdb Viewer (also known as DeepView)

To obtain Swiss-Pdb Viewer go to <http://us.expasy.org/spdbv/>. You'll notice a tool bar to the far left with several buttons. Click the download button. This should bring up a disclaimer. After reading the disclaimer, then accept the licence agreement by clicking the "I agree and want to download Swiss Pdb Viewer now" button and proceed to the next page. Since this tutorial is designed for Swiss-Pdb Viewer for Windows, click on the windows link to download the windows version (I am not sure if this same tutorial will work for the other versions since I have not played with those). The next page should contain the installation procedure for installing Swiss-Pdb Viewer. I actually suggest downloading the self-extracting archive. Download this self-extracting archive to your desktop. Double click on the spdbv37 icon on your desktop and this should bring up a second window. This gives you the option of choosing where to unzip all your files. I suggest unzipping them on your C drive. Wherever choose to store your unzipped files, you should find a folder named **spdbv**. Open this file. You should find an application named **spdbv** with an icon that looks like a wireframe model of an aromatic amino acid. You want to create a shortcut icon to this application and either place it on your desktop or under your start menu for easy access. To create a shortcut icon simply right click on the application named spdbv and a small window should open up. Near the bottom you should see "Create Shortcut". Click this option and a shortcut icon for the spdbv application should appear in this folder. You can take this icon and drag it anywhere. For example you can drag this icon over the start button until the start menu opens, then drag it over the "all programs" link until the "all programs" window appears, and then drop the shortcut icon anywhere in that window.

## Loading in pdb files

Loading pdb files is quite simple. To open a pdb file, click **File -> Open PDB File...** to select a file to open. This can be repeated for any number of structures that you want to appear in your *graphical display* window. Since we will be trying to superimpose multiple structures, go ahead and open up two pdb files in Swiss-Pdb Viewer.

If you need some pdb files to play with, you can either search The RCSB Protein Data Bank [www.rcsb.org/pdb/](http://www.rcsb.org/pdb/) or you can download some pdb files of profilin that I have

stored at [http://microgen.ouhsc.edu/inbre\\_news/](http://microgen.ouhsc.edu/inbre_news/)

## Opening windows

After loading the pdb files that you want to compare into Swiss-Pdb Viewer, we need to make sure our necessary windows are open besides our *graphical display* window.

1. To open the *Alignment* window, click **Window -> Alignment**. This window appears below your protein display window.
  - a. Later we will learn that this window will be helpful after “fitting” two protein structures. This window will highlight for us what amino acids were used to fit the structure that is currently being viewed in the *graphical display* window. The coordinates of the highlighted amino acids were also used in the RMS deviation calculation.
2. To open the *Control Panel* window, click **Window -> Control Panel**. This window appears to the right of the protein display window.
  - a. This window contains details concerning how each amino acid is being displayed on the graphical display. This window allows you to control which groups of amino acids are viewable, which have side chains or not, the color of the amino acids, and their representation style (ribbons, wireframe, or backbone)
3. To open the *Layers Info* window, click **Window -> Layers Infos**. This window appears above and to the right of the protein display window.
  - a. This window contains global information about all protein structures that are loaded in your *graphical display* window. This is useful for quickly making changes to the whole molecule at once, versus making changes to groups of amino acids in the *Control Panel* window.

## Color molecules by ‘Layers’

After loading our pdb files and opening all our windows, we now want to add color to our molecules. There are different ways of coloring any protein. The best way (in my opinion) when dealing with multiple protein structures in a single graphical display window is to give one molecule its own separate color. This will help to the user to distinguish one molecule from another a bit easier.

1. Click **Color -> act on Backbone + Sidechains**
  - a. We need to select what protein representation we want our coloring commands to affect. Swiss-Pdb Viewer loads pdb files in backbone + sidechain representations. We want to make sure that Swiss-Pdb Viewer modifies this representation.
2. Click **Color -> by Layer**
  - a. We now need to select how we want our molecules to be colored. We can

easily color each molecule in our display screen by selecting to color “by Layer”, which corresponds to all the different molecules that are listed in our *Layers Infos* window.

- b. Upon doing this the colors of the sequences in the Alignment window are immediately changed to correspond to the color of the molecule in the graphical display. This same relationship can also be observed with the *Control Panel* window and the graphical display.

### **Create backbone representations of our molecules using only alpha-Carbons.**

Viewing the molecules is still a little confusing. The simplest view for observing structural differences is to view your proteins in a backbone representation. You can use all backbone atoms, or you can use just the alpha-Carbons. The simpler of the two is to view the backbone representation of your protein using only the alpha-Carbons. If we want to see more detail later, we can then add the other backbone atoms as well as side chains.

1. Go to the *Layers Infos* window.
2. Click on the name of the first molecule which is the molecule in the first row.
  - a. This will bring all the amino acids of that molecule to the *Control Panel* window
3. Left mouse click in the column (not on the column name but below it) labeled “side”.
  - a. Unchecking this column will remove all side chains from the selected molecule.
  - b. By left mouse clicking in this column, or any column for that matter, will either check or uncheck all amino acids. You’ll notice that the all amino acids should be checked under the “side” column. Left mouse clicking in that column should uncheck them. Left mouse clicking again will check them.
4. Go back to the *Layers Infos* window
5. Find the column labeled “CA” for the molecule that you are currently working on.
6. Click in the column corresponding to the row for that molecule.
  - a. This will remove all non alpha-Carbons from your structure. This creates the simplest graphical representation for our molecule.
7. Repeat steps 1 through 6 for all structures in the *Layers Info* window.

### **(optional) Give the molecule a better look**

1. Click **Display** -> **Use OpenGL Rendering**
2. Click **Display** -> **Render in Solid 3D**

## Using Iterative Magic Fit

Now that we have our molecules in a backbone representation and colored by layer so we can visually distinguish one from the other, now we can perform some superimposition comparisons. Here we will use **Iterative Magic Fit** to fit our molecules, the *Alignment* window to get a better idea of what amino acids are/aren't aligned, and how to better visualize those regions of misalignment. The difference between **Magic Fit** and **Iterative Magic Fit** is that **Iterative Magic Fit** uses **Magic Fit** for its first fit, and then makes improvements on this fit through several iterations until the RMS deviation is minimized. There is more detailed information about these methods in the handbook.

1. Click **Fit** -> **Magic Iterative Fit**.
  - a. You'll see a *RMS & AutoFit options* window appear.
2. Choose which molecules you want to superimpose from the two drop boxes in the *RMS & AutoFit options* window.
3. Check the "CA (carbon alpha) only" radio button in the *RMS & AutoFit options* window
  - a. Since we are only looking at the alpha carbons, we will only fit the alpha-Carbons
4. Select okay

What is shown to us after we perform an **Iterative Magic Fit**? The *graphical display* window will show you your superimposed protein structures. The *Alignment* window will show what amino acids were used in the fitting process and the RMS deviation calculation. The *Control Panel* window will correspond to the *Alignment* window in that every amino acid that is highlighted in gray in the *Alignment* window will be highlighted in red in the *Control Panel* window.

These windows can be used together to observe structural differences in the alignment. For example, you might observe a region in your alignment where there is a gap. You can highlight this region in your *Alignment* window, which will highlight the corresponding residues in your *Control Panel* window. You can then go to your *Control Panel* window and left click on the "show" column label for both molecules to only display the amino acids displayed in red. You can also turn on your labels to identify the residues that are visualized on your *graphical display* window. You can then zoom in and out, calculate atomic distances, bond angles and dihedral angles using the buttons on the tool bar. (After pressing the button for the calculation you want to perform, just simply follow the directions given to you in the tool bar)

## Explore Fragment Alternate Fits

Swiss-Pdb Viewer looks for alternate superpositions which are displayed on a result list that will pop up on the top left corner of your monitor. This function is very useful to

explore other possible fits. It is also nice because it colors regions of your molecule based on RMS deviation. A high the RMS deviation for an alignment region corresponds to brighter colors such as yellow and red (red is the worse). Again you select these regions you want to explore with out other regions of the molecule being in your way by using the *Alignment* window and *Control Panel* window.

1. Click **Fit -> Explore Fragment Alternate Fits**
  - a. You'll see a *RMS & AutoFit options* window appear.
2. Choose which molecules you want to superimpose from the two drop boxes in the *RMS & AutoFit options* window.
3. Check the "CA (c arbon alpha) only" radio button in the *RMS & AutoFit options* window
  - b. Since we are only looking at the alpha carbons, we will only fit the alpha-Carbons
4. Select okay

### **Calculating RMS**

The RMS deviation of a fit is always reported after every superimposition at the top of the screen under the tool bar in red letters. It is calculated using the atoms you specified in the *RMS & Auto Fit* window. You can also calculate the RMS deviation on a specific region between both superimposed structures. All the amino acids you choose to use in the calculation must be highlighted in red in the *Control Panel* window. This can be done by clicking and dragging your mouse across the desired amino acids in the *Control Panel* window or *Alignment window* to select amino acids.