

## Visualization and analysis tutorial.

### Outline of document:

1. *View Manipulation*
2. *Selecting an manipulating specific parts of the molecule*
3. *Making high –quality photos*
4. *Colouring objects*
5. *Examine the beta-sheet*
6. *Examine the binding of the cesium ion*
7. *B-factor putty*
8. *Molprobit*
9. *PISA*

### PyMol tutorial

Adapted from: <http://ibis.tau.ac.il/twiki/bin/view/Bioinformatics/ProteinVisualization>

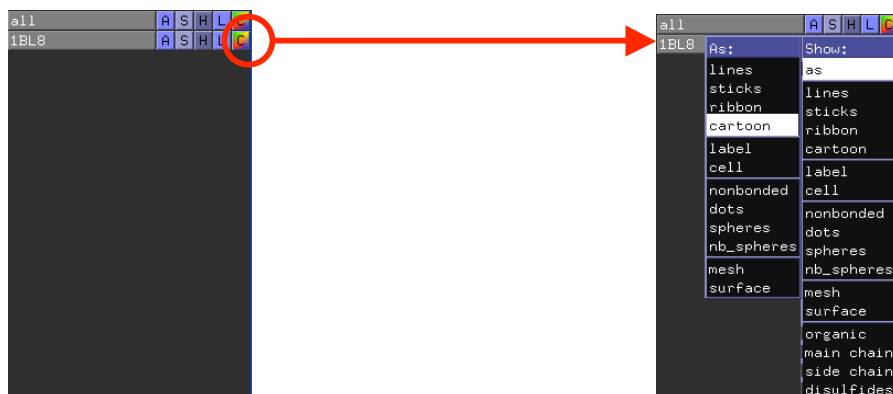
PyMol contains many features but we will only cover the most basic ones. For a more advanced tutorial, see the PyMol documentation at:

<http://pymol.sourceforge.net/newman/user/toc.html>

### Part 1: View Manipulation

1. Run PyMol
2. Download and load '1ULZ.pdb' (<http://www.pdb.org/pdb/home/home.do>)
3. Identify the different parts of the screen. There are two windows – the external GUI window and the internal GUI window. The internal window contains the viewer, which displays the molecule, and the command line.
4. Manipulate the view with the mouse. Dragging with the left button rotates the molecule. Dragging with the right button zooms in and out. Dragging with the middle button moves the molecule.
5. You can manipulate objects in the internal GUI. There are currently two objects – “all” – which contains all the viewed molecules (currently there is only one molecule) and “1ULZ” which contains the molecule loaded from the file “1ULZ.pdb”.  
To manipulate an object, we use the letter icons near its name (A – Action, S – Show, H – Hide, L – Label, C – Color).

Change the representation of the object to “Cartoon” using Show->As->Cartoon



## Visualization and Analysis Tutorial

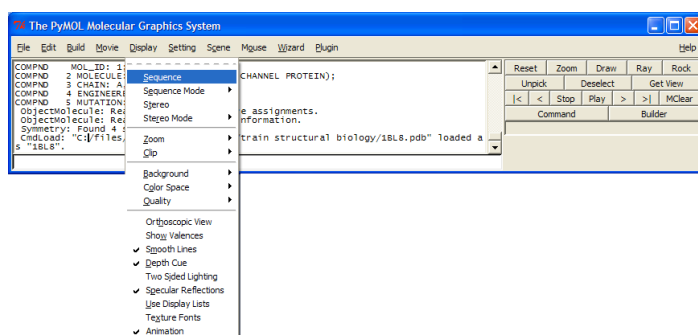
Try viewing the molecule in several representations (lines, sticks, ribbons, cartoon, spheres, surface)

- Notice that when you rotate the molecule, it rotates around some atom, called the **center atom**. You can change the center atom by clicking on an atom with the middle mouse button.
- If you want to investigate the area of the center atom, sometimes the rest of the molecule gets in the way. You can darken everything around the center atom using the mouse wheel.

Now is a good time to get comfortable with using PyMol. Try playing with the controls a little, and view the molecule from several angles and in different representations.

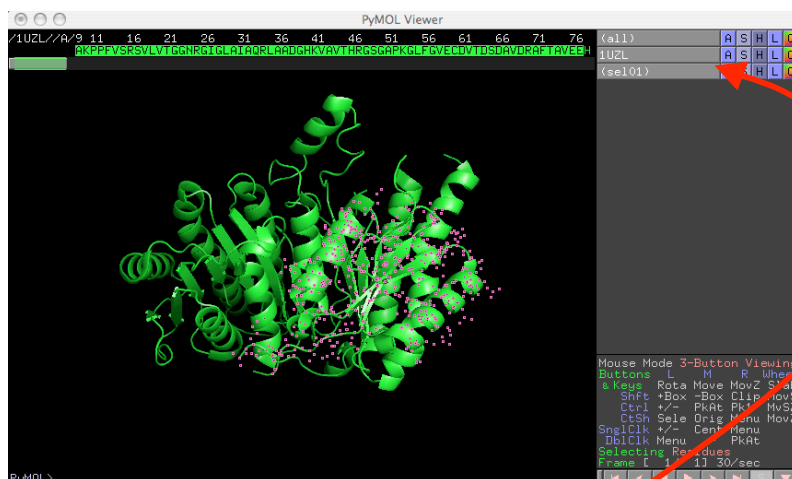
### Part 2: Selecting and manipulating specific parts of the molecule

- The easiest way of selecting specific parts of the molecule is by using the sequence. Choose Display->Sequence in the external GUI menu.



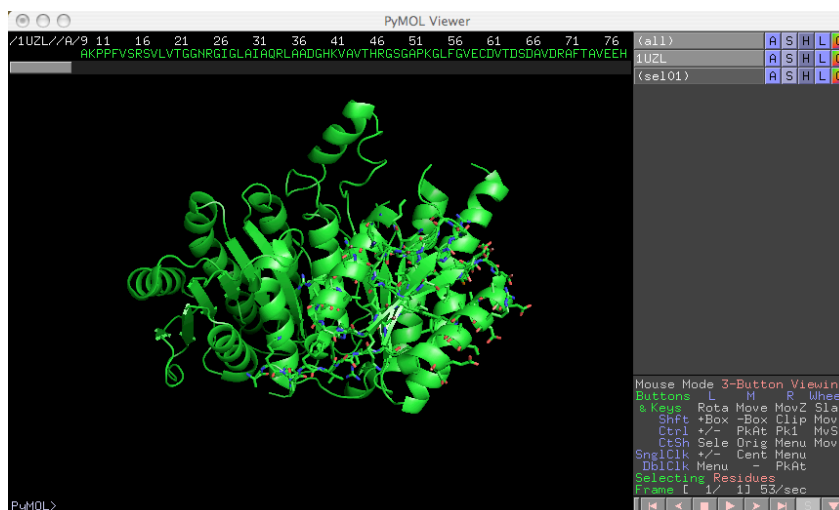
You can now see the amino acid sequence on the top of the viewer:

- You can select specific amino acids by clicking on them. You can also select a range in the sequence by clicking the first residue, and then shift+click on the last residue. Your selection will be indicated on the structure (in pink dots). Try selecting the first chain of the molecule:



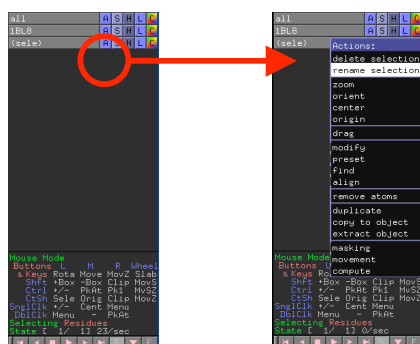
- Notice in the object list that a new object “(sele)” was added. This object represents your current selection (the first chain). You can manipulate it with the buttons next to the object. For example, change its representation to sticks (S -> As -> Sticks)

## Visualization and Analysis Tutorial



(Before making a new selection, disable the current selection by clicking on the black area in the viewer), or the "Deselect" button in the external GUI.

- For convenience, you can give a different name to the selection, so you can easily manipulate it later. Select the first chain again (using the sequence) and change it name to "chain1" by pressing "Action -> Rename Selection" and typing "chain1".

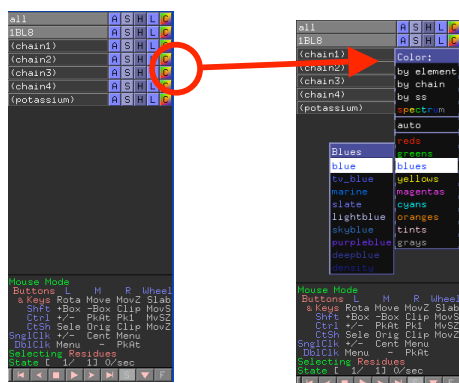


- Change the representation of chain1 back to cartoon
- Now give names to all the other chain ("chain2").
- Also select the 4 cesium atoms (Cs) and give the selection a name ("cesium")

You should end up with 5 different objects (including "all" and "1UZZ"):

You can enable and disable each selection by clicking on the object name (and see where it is located on the structure in the viewer)

- You can color an object using the "Color" menu. Try coloring the first chain in blue:



Do the same for the other chain, coloring it red.

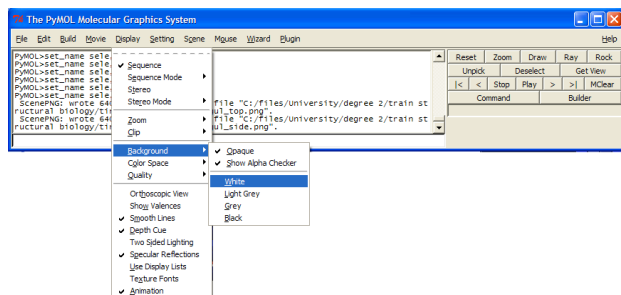
# Visualization and Analysis Tutorial

9. Show the cesium ions in sphere representation.

## Part 3: Making high-quality photos

Molecular visualization programs such as PyMol are often used to make high-quality photos for use in publications.

1. Change the background color to white, with Display -> Background -> White on the external GUI menu:



A white background usually looks much better when printed or attached in documents. It's also important if you don't want to finish the black ink when printing the image...

2. To make the high-quality image, right click anywhere in the background area of the viewer (to open the "Main Pop-Up") and choose "ray":
3. Finally, to create the image file, choose File -> Save Image in the external GUI menu.

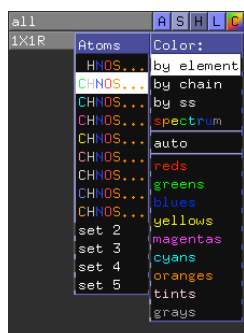
## Part 4: Coloring objects

1. View the molecule in sticks representation
2. PyMol has some useful color commands. First try "Color by chain" which colors each chain in a different color. This view usually helps identify the interaction between the different subunits.

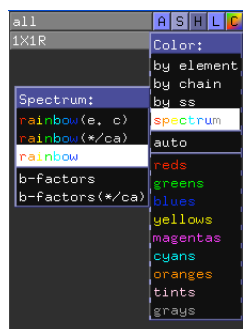


3. Try "Color by element" which colors each element in a different color. This view helps us identify chemical interactions.

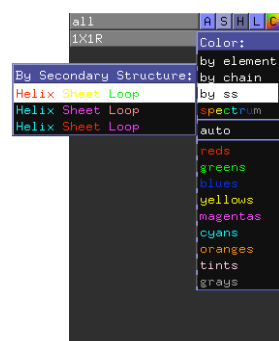
## Visualization and Analysis Tutorial



4. View the molecule in cartoon representation
5. Try “Color Rainbow”. This helps us trace the molecule’s backbone.



6. Try “Color by ss”. This shows us the topology of the protein (division to secondary structure).



### Part 5: Examining the beta-sheet

1. As can easily be seen using the cartoon representation with “Color by ss”, the structure contains a 7-strand  $\beta$ -sheet. Select the  $\beta$ -sheet, and name your selection “sheet”.  
(Select the sheet using the sequence display. It should be easy because the sequence is colored the same as the structure, so just select all the yellow residues).

2. Show only the  $\beta$  sheet:

(1UZL) hide -> everything

(sheet) show -> as -> cartoon

Notice that the  $\beta$ -sheet is parallel. Also notice that it is twisted into half a barrel ( $\beta$ -sheets are usually twisted this way).

3. Use (sheet) Action->Orient, to zoom and orient the view near the beta sheet.

## Visualization and Analysis Tutorial



4. We can use PyMol for finding polar contacts in the molecule. As an example, let's examine the polar contacts between the strands of the  $\beta$ -sheet. Use:

(sheet) Action -> Find -> Polar Contacts -> within selection

This adds a new object called "sheet\_polar\_conts" and shows the polar contacts in yellow. It's pretty hard to see them with this color because the strands are also yellow, so paint the contacts in red:

(sheet\_polar\_conts) Color -> Red -> Red

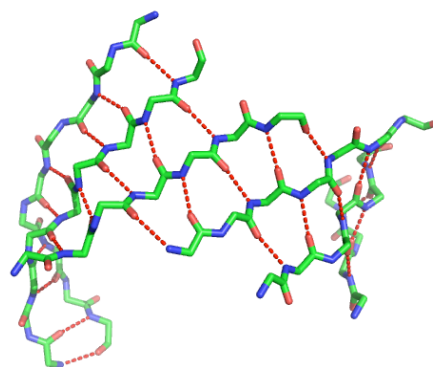
5. This looks a little weird, because you can't see the contacting atoms. To get a better view:

(sheet) Hide -> Cartoon

(sheet) Show -> As -> Sticks

(sheet) Hide -> Side Chain

(sheet) Color -> By Element



And now we can clearly see the web of polar contacts stabilizing the  $\beta$ -sheet.

6. Now let's measure the rise per residue of these  $\beta$ -strands.

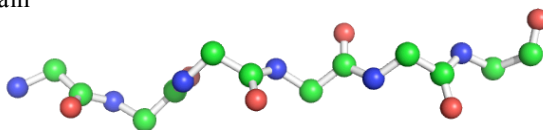
Select one strand of the beta-sheet, and name it "strand". Show only the main chain in ball-and-stick representation using the following commands:

(strand) Action -> Orient

(all) Hide -> Everything

(strand) Action -> Preset -> ball and stick

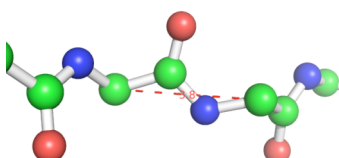
(strand) Hide -> Side chain



Measurements are done using the "Measurement Wizard". To access it, use Wizard->Measurement in the external GUI menu. This will open the measurement box in the internal GUI:



After it opens, click on two consecutive  $C_{\alpha}$  atoms to measure the distance between them.



## Visualization and Analysis Tutorial

The rise changes a little from case to case, but it should always be around 3.5Å.

- Next, we measure the dihedral angles between two residues in the sheet ( $\Phi$  and  $\Psi$ )

To measure the angles, click on “Distances” in the Measurement box and change it to “Dihedrals”.

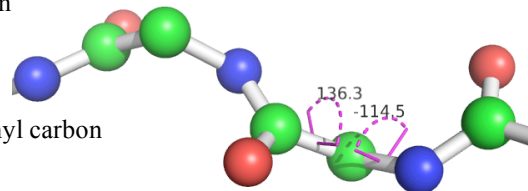


Dihedral angles are defined for 4 atoms, so you are expected to choose the 4 atoms for measuring the angle. To measure the  $\Psi$  angle, you need to click:

Nitrogen → Carbonyl carbon → Alpha carbon → Nitrogen

To measure the  $\Phi$  angle, you need to click:

Carbonyl carbon → Alpha carbon → Nitrogen → Carbonyl carbon



The angles you receive should be around the usual values for a  $\beta$ -strand, which are  $\Phi=-125^\circ$   
 $\Psi=+125^\circ$

- Press “Done” in the measurement box to stop measuring.

### Part 6: Examining the binding of the cesium ion

- Reset the view for the molecule (either exit PyMol and then run it again, or use File->Reinitialize in the external GUI menu, then load 1UZL again.)
- Select a cesium ion and name your selection “ce”
- To examine the binding site for cesium, we can find the residues that are close to the ion using the following command:

Type: “Select near\_ce, byres ce around 5”

This creates a new object called “near\_ce” which contains all the residues which are within a 5Å radius to the cesium ion.

- Get a good view of the ce ion and the residues surrounding it:

```
(1UZL)Hide->Everything  
(ce)Show->As->Spheres  
(ce)Color->Magentas->Purple  
(near_ce)Show->As->Sticks  
(near_ce)Action->Orient
```

- There are also water molecules around the ion, but they can’t be seen in the sticks representations. To show the water molecules, use:

```
(near_ce)Show->nb_spheres
```

## Visualization and Analysis Tutorial

This will show the oxygen atoms of the water molecules as small red spheres

6. To see the identities of the residues neighboring magnesium, use:

```
(near_ce)Label->residues
```

This adds a text label for each residue in the object. To be able to see the labels better, type in the command line:

```
set label_color, red
```

7. To see the contacts around the cesium ion:

```
(near_ce)Action->Find->Polar Contacts->Within Selection
```

Using PyMol we can clearly see that the cesium ion is being held in place by a few contacts, some direct and water molecules mediate some. Using this information we can predict, for example, the effect of some mutations on the binding of cesium.

### Part 7: B Factor Putty

The B-factor of an atom is an experimental value that reflects its fluctuation in the crystal. In other words, it reflects how flexible this atom is in the crystal structure, and probably also in the biological structure.

PyMol can give a nice view of the B-factors with:

```
(1UZL) Action->Preset->b factor putty
```

In this view, the thicker the backbone is, the more flexible it is. As can be expected, the loop areas that point toward the aqueous environment are the most flexible.

Using chimera

- <http://ibis.tau.ac.il/twiki/bin/view/Bioinformatics/ProteinVisualization>
- 8. Molprobit ( <http://molprobit.biochem.duke.edu/> )
- 9. PISA-Protein Interfaces, Surfaces and Assemblies ( [http://www.ebi.ac.uk/msd-srv/prot\\_int/pistart.html](http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html) )