PyMOL Exercise I

Hand-in details

Important: The results of this exercise have to be handed in electronically (CampusNet: PyMOL hand-in #1) no later than Wednesday next week. You only need one hand-in per group – but remember to put the names of all group members on. Group members not mentioned on the exercise will not be credited and approval of this exercise is a prerequisite for the exam!

Purpose

The purpose of this exercise is to make images in PyMOL of either hemagglutinin or neuraminidase similar to the ones you will need for your final project. Here, you will need to fetch the model (either from CPHmodels or HHpred) that you created in the previous exercises to start working.

You will generate four images:

1. An overview image comparing the model with the template structure.
2. An overview image showing the biologically relevant multimeric state.
3. An overview image with interesting features highlighted.
4. A zoomed image of the binding pocket/active site with some ligand or drug taken from a real HA or NA structure.

For each image you have to provide a short figure legend to explain what the image shows. You will also need to briefly explain how the image was made (such as choice of selections and representations). Although the log function in PyMOL can be useful for this, I do not want the raw log output!

Some suggestions and a warning before you start:

- The warning first: Do not name your protein models “model” inside PyMOL as this will get PyMOL confused. Use model1 or something similar.
- Try to keep the images simple – too many details will obscure the message.
- Play with colours.
- Remove unnecessary information (select and hide it).
- For print use a white background for your images.
- Ray-trace images for a nicer appearance (“Ray” button on the top right).
The exercise
You will make four images of either your HA or NA model.

1. **Comparing the model with the template: An overview image with differences highlighted.**

For the first image, show differences in structural features between your model and the template showing *only the protein backbone*. Highlight any feature that you find interesting. It could be simply the secondary structure, e.g. with different parts of the structure coloured differently according to sequence numbering, amino acid residue type, secondary structure type etc. It might show glycosylation sites, cleavage sites, highlight missing parts (showing where they should have been), demonstrate overall flexibility (Color→Spectrum→b-factors) or surface electrostatics (Actions→generate→vacuum electrostatics→protein contact potential). Or it might simply highlight the position of the active site/binding pocket relative to the overall structure, transmembrane segments etc.

If you made a HA model, it most likely consists of two independently modeled chains. Align each of these to the appropriate parts of the template. Remember to include chain names and possibly residue numbers to get things aligned correctly.

The general align command is:

```
align model1, template1
```

and will move model1 onto template1. The image should highlight missing parts such as loops and transmembrane segments by showing where they should have been. Use colours or add arrows (or similar) to the image after it has been produced in PyMOL.
2. **The biomolecule: An overview image showing the biologically relevant multimeric state.**

For the second image, you should assemble the biomolecule and show this in a cartoon representation. Remember that both HA and NA are multimers (HA is a trimer, NA is a tetramer). To show this for the overview structure you will have to align your model to a template structure in the multimeric state. Depending on your choice of template this could be the actual template used, but you may have to look in the PDB for alternative templates in case yours was not multimeric. Simply make copies of your model(s) (Actions→duplicate object) and align each copy in turn to the different subunits of the template. Again, if you made a HA model, it most likely consists of two independently modeled chains. Align each of these to the appropriate parts of the template. Remember to include chain names and possibly residue numbers to get things aligned correctly. See align command above.

**A general caution:** Models consisting of several independently modeled parts are unlikely to be accurate in the interfaces. This goes for the generation of multimers as well as interfaces between the individual chains of a model (here HA).
3. **Interesting features.**

For the third image, highlight any feature that you find interesting on the assembled biomolecule. It could be simply the secondary structure, e.g. with different parts of the structure coloured differently according to sequence numbering, amino acid residue type, secondary structure type etc. It might show potential glycosylation sites, cleavage sites, highlight missing parts (showing where they should have been), or demonstrate surface electrostatics (Actions→generate→vacuum electrostatics→protein contact potential). Or it might simply highlight the position of the active site/binding pocket relative to the overall structure, transmembrane segments etc.
4. **The binding pocket/active site: Showing how ligands or drugs will bind.**

The fourth and last image is meant to show a close-up of the active site/binding pocket using surfaces. It should demonstrate how a drug or the natural ligand fits in the active site/binding pocket. Do not show the individual residues involved in ligand binding (these are the topic of the next hand-in exercise). Again, a surface electrostatic plot could be interesting (see above), but in general regular surfaces are better suited to illustrate the shape of a pocket.

**A few comments regarding surfaces:**

- Surfaces are generated as part of an object. This means that defining a surface from a selection (which is not the whole object) will only show a surface for those parts of the object included in the selection. Such surfaces are likely to be partial, i.e. with holes or consisting of patches. To get a closed surface for a sub-selection, simply turn the selection into an object (Actions→extract object).
- Surfaces will take on the colour of the corresponding object (or selection).
- Surfaces can be made transparent. Go to the main menu: Setting→Transparency→Surface→[value]. Alternatively, type on the command line:

```
set transparency, 0.5
```

in this case to get a transparency of 50%.
- Try using two-sided lighting (Display→Two Sided Lighting).
- Remember that you can use the scroll wheel of the mouse to control the thickness of the slab, i.e. the slice of the structure currently being viewed. This is very useful for viewing inside pockets. To reset it, simply type

```
zoom
```

or press the “Zoom” button on the upper right of the command window.

**Getting a drug/ligand:**

To do this you have to “steal” a ligand from a real structure. Find a structure in the PDB with a suitable ligand bound in its active site. Align this real structure to your model and then do one of the following to graft the ligand into your model:

a) Make an individual object of the ligand and show this together with your model.

b) Alternatively, select the ligand, switch off all representations of the real structure that came with the ligand (Hide→everything) and show only a representation of the ligand with your model, e.g. as sticks.

To get the best possible fit between your model and the ligand-containing structure, align the model to the structure by selecting residues around the active site. For a
structure with residues 120-200 constituting the active site domain, this would look like the following:

    align model1 and residue 120-200, real_structure

This might seem unnecessary, but it will assure you the best fit between your model and the ligand. Here, an offset of just a few Å will make a world of difference!

**Before you leave**
Remember to save your work for next week!

**Handing in**
Don’t forget to explain how you made your image, and do remember a small figure legend for each image explaining what is seen.

**Useful links**
- PyMOL home:  
  - [http://www.pymol.org/](http://www.pymol.org/)
- PyMOL manual:  
  - [http://pymol.sourceforge.net/newman/user/toc.html](http://pymol.sourceforge.net/newman/user/toc.html)
- PyMOL Wiki:  
  - [http://www.pymolwiki.org/](http://www.pymolwiki.org/)
- PyMOL settings (documented):  