1 PyMOL Practicum

Visualizing very tiny things.

1.1 Where can I get structures?

- Download directly from *pdb.org*, then go to $File \rightarrow Open$
- Directly in PyMOL: $Plugin \rightarrow PDB\ loader\ service$

TASK:

1. Load the structure of HhaI DNA cytosine-5-methyltransferase, S-adenosyl-L-homocysteine, 13-mer with 5-fluorocysteine at target site (PDB ID 1MHT) into PyMOL.

1.2 What is PDB file format?

- HEADER lines store "metadata" about the structure (method used to solve it, organism, etc.)
- ATOM and HETATM lines store Cartesian coordinates of atoms
- TER lines indicate breaks between chains
- CONNECT lines indicate bonds (these are only necessary for nonstandard atoms/residues)

TASK:

1. Open "1mht.pdb" in a text editor. NOTE: MS Word is *NOT* a text editor. A text editor will be something like "Notepad" on Windows.
2. Go to the first *ATOM* line. Can you figure out what each column stores?
3. Delete every line but the *ATOM, HETATM*, and *TER* lines, save this as a new file, and re-open in PyMOL. Does the structure look the same?

1.3 How do I navigate in 3D?

- Left-click/drag: free rotate
- Right-click/drag: zoom in and out
- Middle-click/drag: move center of view
- Scroll-wheel: expand and contract "clipping plane"
- Left-click on the object to select. The name of the selected atom will appear in the "console" at the top.

- Right-click to open object menu.
- "Center" will center the view on the selection.
- "Zoom" will zoom in on the selection.

TASK:

1. Load 1MHT.
2. Select Thymine 421 from chain C.
3. Zoom in on that residue.
4. Reorient the view to see its context. What base is T421 "base-stacking" with?

1.4 How do I select stuff?

- Left-click on the object. The green "Selecting" entry (bottom right of viewing panel) indicates what the click will select (atom, residue, chain, etc.)
- Type a command:

  $$\textit{sele resid } 421$$

  These selections can be compounded:

  $$\textit{sele resid } 421 \textit{ and chain } C$$

- Use the sequence tool. Go to:

  $$\textit{Display} \rightarrow \textit{Sequence}$$

  and then click on the residue of interest, which will appear at the top.

TASK:

1. Select the entire protein chain.
2. Select one atom on base T421 from task 1.3.

1.5 How do I change the molecular representation?

- The S column on the right lets you change what you Show. "$S \rightarrow As$" will make the object have *only* that representation. $S \rightarrow sticks$ will add the "sticks" representation on top of existing representations.
- Type a command:

  $$\textit{show cartoon, all}$$

- You can change the color with the C column on the right.

TASK

1. Simultaneously display the 1MHT structure from task 1.3 as cartoon and lines.

2. Make the protein chains in the structure your favorite color.

1.6 How do I save?

- *File* → *Save session...* allows you to save whole PyMOL session (selections, colors, representations, etc.) as a *.pse* file.

- *File* → *Save molecule...* allows you to save out the coordinates as a *.pdb* file.

- *File* → *Save Image As* allows you to save out a snapshot as a *.png* file.

  - NOTE: To make the image look good, you should first hit the the *Ray* button (top right), which will "Ray Trace" the 3D model. Then save the image out.

TASK

1. Save your current session out as a .pse file.

2. Find a cool orientation for your molecule, ray trace it, and save out a .png file. You've just made your new Desktop background.

1.7 How do I measure structural stuff?

- Use the wizard:

  - Zoom in/center the residues you want to characterize.
  - Go to: *Wizard* → *Measurement*. The right panel will now have a "*Measurement*" box
  - Click on "*Distances*," which will allow you to select different things to measure. (If you're interested in distance, you can skip this step as distances are already selected.)
  - Left click on the atoms that define what you measure. (For example, for a distance, click on two atoms; for a Euclidean angle, click on three atoms; for a dihedral angle, click on four atoms).

TASK

1. Measure the distance between T421 and the nearby base you identified in task 1.3.

1.8 How can we estimate the structural effect of a mutation?

- Use the wizard:
  - Zoom in on the residue you want to mutate.
  - Go to $Wizard \rightarrow Mutagenesis...$ The right panel will now have a "$Mutagenesis$" box.
  - Left click on the residue you want to mutate.
  - Click on "$No\ mutation$," which will bring up a menu allowing you to choose the thing you're mutating to.
  - Select the amino acid you want to jam in.
  - Use the arrow keys to walk through different "rotamers" of the amino acid, selecting the one with the fewest clashes.
  - Click "*Apply*" in the mutagenesis box and then "$Done$."

TASK:

1. In a new PyMOL session, download the structure of *Staphylococcal nuclease* (1STN).
2. Mutate Valine 66 to Lysine.
3. Can you find a rotamer that doesn't clash with the rest of the protein?
4. Do you think that this structure is reasonable?
5. What do you think the charge state of Lys-66 would be at pH 7?

1.9 How can I align two structures?

- Load in both structures and type $align\ MOVE,\ REF$ where $MOVE$ is the name structure to move and REF is the name of the structure to align $MOVE$ to.

TASK:

1. Download the structures of human γD-crystallin (2G98) and human γB-crystallin (2JDF).
2. Align 2JDF to 2G98.
3. Display them both as cartoons.
4. Why do you think there are non-overlapping chains of 2G98 floating out in space?

1.10 How do I calculate more interesting things than just structure?

- Polar contacts:
  - Go to the *A* column for the structure of interest on the right.
  - Go to "*Find* → *Polar contacts* → *selection you want*"
- Electrostatics:
  - Go to the *A* column for the structure of interest on the right.
  - Go to "*Generate* → *Vacuum electrostatics* → *protein contact potential*"
  - WARNING: the actual electrostatic field is total crap. This is *only* for identifying patches of more positive (blue) or more negative (red) residues.

TASK:

1. Open a new PyMOL session and load in 2JDF and calculate its electrostatic surface.
2. How would you describe the charge distribution?
3. Open a new PyMOL session, load in the structure of the *Y. pestis* virulence factor YopM (1G9U), and calculate its electrostatic surface.
4. How would you describe the charge distribution?
5. What do you think happens to the "stability" of 2JDF as you add salt? What about 1G9U? Why?