PyMol Assignment 4: Structure Manipulation and Measuring Protein-Protein Contacts

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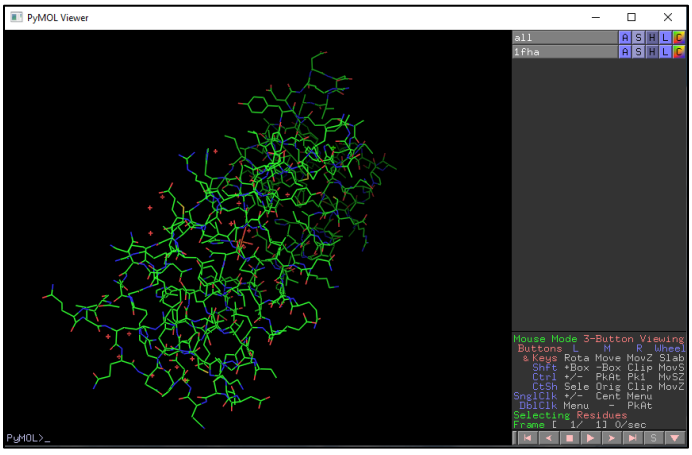
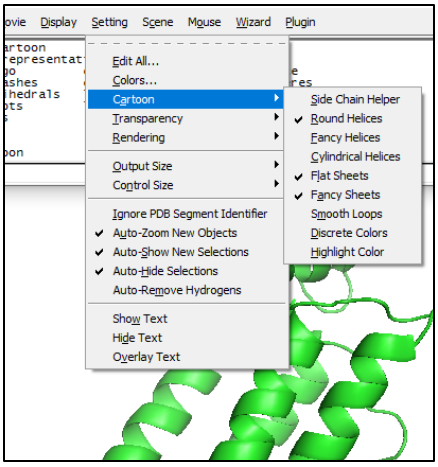
Before you start copy (all together) and paste the commands here into the Pymol command line

```
# delete all objects and reset pymol
reinitialize
# set the background color to white
bg_color white
# make the background transparent for ray trace
set ray_opaque_background, 0
# set the ray trace mode
# normal color
set ray_trace_mode, 0
# normal color + black outline
#set ray_trace_mode,  1
# black outline only
#set ray_trace_mode,  2
# turn off shadows during ray trace
set ray_shadows, 0
# set the mouse mode for laptop.
config_mouse one_button
# get rid of double bonds and skinny bonds to H
set stick_h_scale, 1
set valence, 0
# high quality surfaces
set surface_quality, 3
```

1. Load the crystal structure of human H ferritin, PDB structure file 1FHA. This can be done with the fetch command (as in assignment 2), or by retrieving the file from the PDB and using the load comand. The monomeric building block of the ferritin cage will appear. *Note that ferritin is a 24-mer and forms a protein cage. Due to the high degree of symmetry within the structure (octahedral, space group F432), solving the structure of the monomer is sufficient to recapitulate the entire 24-mer cage. [*note that in more recent versions of Pymol the default display is cartoon, not stick. The background will be white.*]

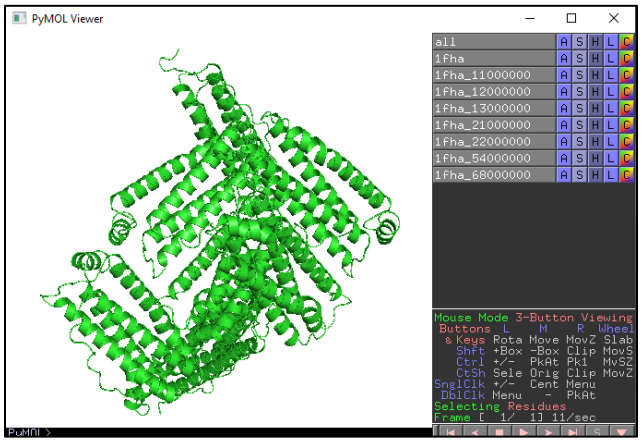




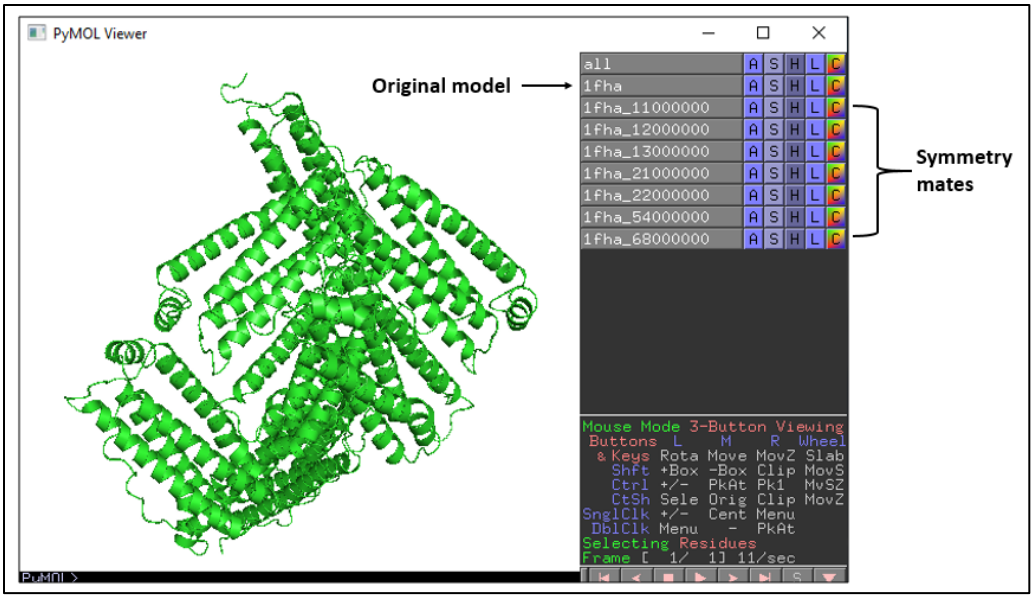
2. [*this step is uneccessary in the most recent version of Pymol which displays by cartoon by default.*] Show the structure as a cartoon for easy visualization. Using the script command, type "hide everything, 1FHA", which will hide the structure, then type "show cartoon, 1FHA". (If you type "show cartoon, 1FHA" without first hiding the structure, cartoon view will overlay with the stick view.) The structure will appear as a cartoon rather than sticks. Alternatively, the Show Command Button "S" can be used to show the structure as a cartoon, with S→As→Cartoon.

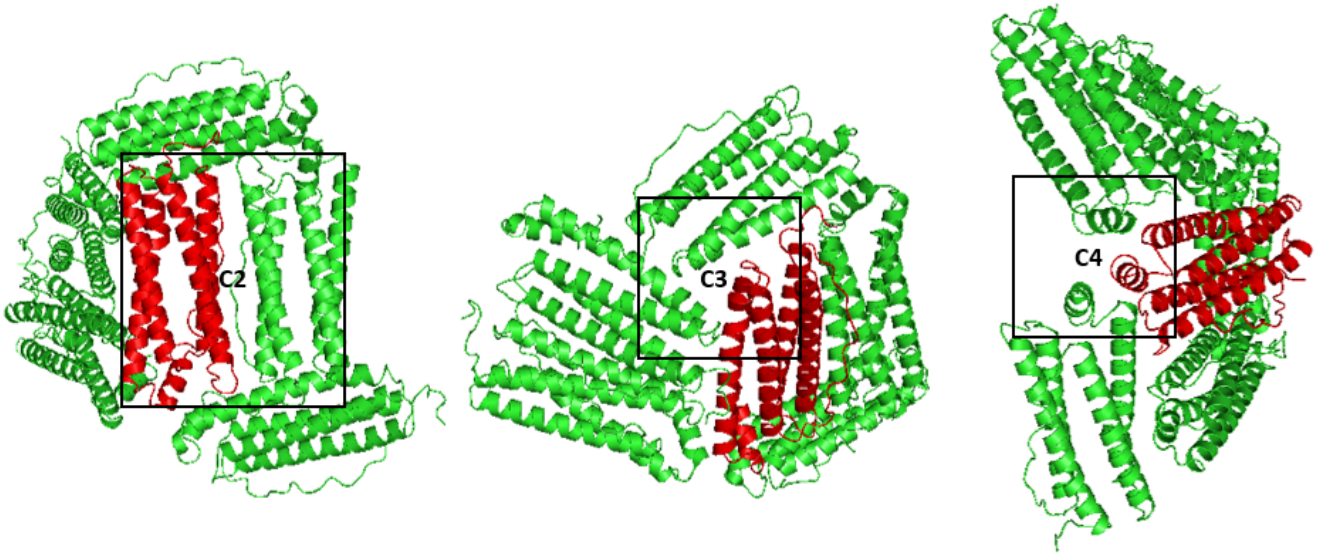
3. There are several options for visualizing alpha helices. Ferritin is a 4-helix bundle, and depending on the application, one may want to view the helical bundle in different formats. Looking at the menu bar at the top, use Setting→Cartoon→, try visualizing the ferritin monomer with Round, Fancy, or Cylindrical helices. Chose the Fancy setting for now.

4. The structure you are looking at is a monomer, but functionally, the ferritin forms a multimer cage. We want to investigate monomer-monomer interactions within the C2 dimer interface of ferritin. Due to symmetry, there are distinct C2-, C3-, and C4-symmetric interfaces. To see more of the protein cage, we need to generate, using symmetry, more of the assembly. To do this, look to the right of the molecule display and use the Action Command Button "A". The sequence of clicks is A→Generate→Symmetry Mates→. At this point, there is the option to choose the distance from the monomer building block that we want to search for crystallographic symmetry mates. Let's start with the shortest search distance, or →Within 4 Å. A number of symmetry-related monomers should now appear. <u>This might look confusing because there are a lot of atoms here, but don't be intimidated!</u>



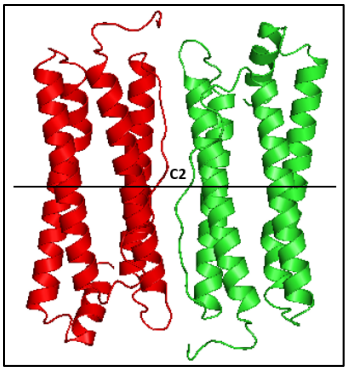
Annotated in the panel on the right are objects containing the original model and seven symmetry-generated monomers. To easily identify the original monomer in the mess of symmetry mates, try coloring it red using the Color Command Button "C". Now, it should be obvious which of the monomers was the original. To clarify your view, remove two of the symmetry mates that are not part of the same ferritin cage), 1fha_54000000 and 1fha_68000000. This can be done with the Action Command Button "A" with the following sequence: A→Delete Object. Try moving the rotating the remaining objects around and look for the different symmetries [C2 (2-fold symmetry), C3 (3-fold symmetry), C4 (4-fold symmetry),] which I highlight below. *Note that C4 is incomplete. Also note that the full cage can be generated by expanding out the distance from the original





monomer that symmetry mates are sought.

5. Click through the symmetry mates (turn them off and on) to determine which completes the C2 axis with the original (red) monomer. This turns out to be 1fha_22000000. Delete all the objects except 1fha and 1fha_22000000 so we can easily explore the protein-protein interactions at the two-fold, C2 dimer interface. Again, to delete symmetry mates, use the Action Command Button "A" with the sequence: A→Delete Object. You should now have the ferritin dimer. Rename the two monomers MonomerA and MonomerB using the Action Command Button "A" with the sequence: A→Rename Object. Change the color of MonomerA back to green using the Color Command Button "C".



6. The C2 ferritin dimer interface is complex, with participation of 2 alpha helices from each monomer plus a long loop from each as well. The monomers are arranged in an antiparallel fashion, such that the top half of the interface is repeated on the bottom half. An understanding of one half will thus be sufficient for a total understanding of the interface.

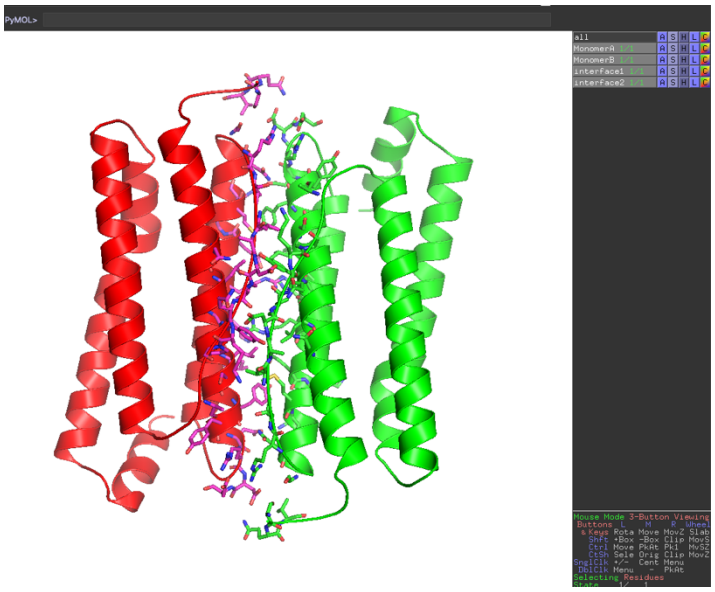
In the command line type (copy and paste):

create interface1, byres((MonomerA) within 4 of MonomerB)
create interface2, byres((MonomerB) within 4 of MonomerA)

These commands create objects that contain all of the amino acids of MonomerA that are within 4 Å of MonomerB, and vice versa.

Use the H and S buttons to hide everything for interface1 and interface 2, then show them both as sticks.

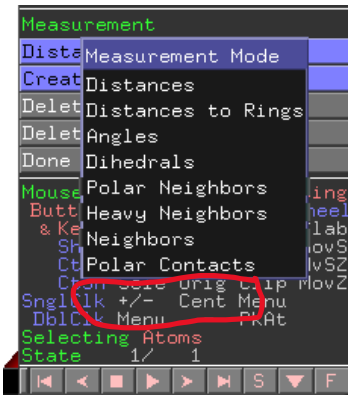
Use the C button to color objects interface1 and interface 2 by element, with a different coloring scheme for each. When you are done your window should look that on the right.



7. Explore the interface and find which residues make key contacts with one another. For example, let's look at Aspartate 42 of MonomerA. To find this residue quickly, type

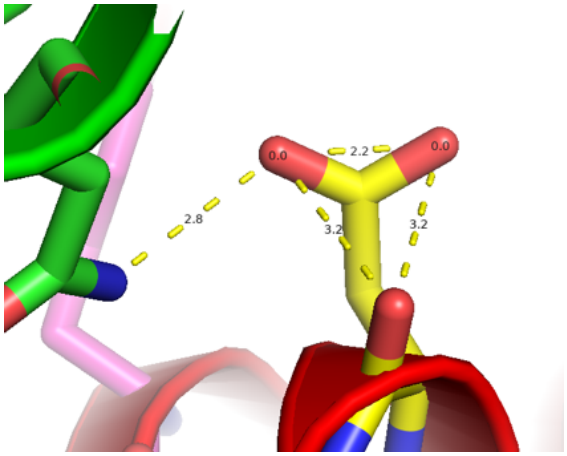
color yellow, name C* and resi 42 and interface1
center resi 42 and interface

The yellow color is just to help keep track the amino acid that we are interested in. To determine if Asp42 makes contacts with other residues at this interface, the use the Measurement tool. At the top of the screen choose Wizard→Measurement. The default is a Distance measurement. Clicking on Distance allows



you to scroll through the types of measurement available. These include Distances, Angles, Dihedrals, Polar Neighbors, Heavy Neighbors, and Neighbors. Let's start by looking for Polar Neighbors of Asp42. This will give us an idea if Asp42 participates in hydrogen bonding/salt bridge/ionic interactions, either intra- or inter-monomer.

Once Polar Neighbors is selected, the message on screen switches to "Please click an atom". Choose one of the carboxyl oxygens of Asp42, and then choose the other. Asp42 of MonomerA makes contacts with its backbone oxygen (intramolecular interactions). These are not hydrogen bonds since both the sidechange and the C=O are hydrogen bond acceptors. There is a hydrogen bond with Asn74 from MonomerB. This hydrogen bond helps stitch together the dimer interface of ferritin. To determine which atoms are interacting, right click the atom of interest, and the identity of the atom will be given (atom name/type, monomer/chain it belongs to).



8. What are some of the other Polar Neighbor interactions that are key to this interface?

9. Using the Neighbors or Heavy Neighbors measurment tools, identify any hydrophobic interactions at this interface that contribute to holding the ferritin monomers together.