Analyzing the structure of ubiquitin in PyMOL

In the following guide, all PyMOL commands are printed in boldface. You can type these commands into either of the PyMOL command lines.

Some tips

If you ever want to re-center the view on the protein, use **orient 1UBQ** .
You can activate and deactivate molecules by clicking on them in the "names panel".
You can perform almost all commands using the buttons in the names panel as well.
Clicking on a residue in the sequence will select it.

Getting Started

Load the structure: **fetch 1UBQ**

The default representation in PyMOL is the "lines" representation. You will see some green, red, and blue lines on a black background. You may not find this visually appealing.

background color: **bg_color white** (note: no comma)
Show sequence: **set seq_view, 1** (or click display → sequence in GUI)
Hide lines: **hide everything, 1UBQ** (note: comma)

The protein structure will disappear and you will be left with a white background. The structure is still loaded, it is just not visible. We can now show the structure in different representations. The best view to get an overall view of the structure is the so-called "cartoon" representation. Individual representations such as sticks, cartoon, surface, etc can be turned on or off with **show** and **hide**.
Show cartoon: **show cartoon, 1UBQ**

The cartoon representation is the most common representation reported in the literature. The overall protein fold and secondary structures are most apparent in this representation. Nonetheless, it can get busy for proteins larger than ubiquitin.

Now analyze the secondary structure: which elements do you see? Which amino acid regions do they span? (hint: clicking on an amino acid in the viewer will bring up some information in the external GUI log and also highlight it in the sequence)

Structure and function

The proposed mode of attachment of ubiquitin to target proteins is linkage of the ubiquitin C-terminus to either the N-terminus or lysine side chains of the target protein. Where is the ubiquitin C-terminus located in the structure? Is its location consistent with this proposal?

Ubiquitin contains seven lysine residues. Two of these residues are known to be important in linking ubiquitin chains to each other. Let's first look at all the Lys residues in ubiquitin.

Select all lysines: **select lys, 1UBQ and resn lys**
Show lysines as sticks: **show sticks, lys**

These commands will show all Lys residues in stick representation. By default, oxygen atoms are colored red and nitrogen atoms blue. Carbon atom coloring changes with each molecule loaded. From this view, can you determine which Lys residues are involved in linking ubiquitin chains? Why or why not?

It turns out that the most important Lys residues are Lys48 and Lys63. Select just these residues.

Hide other lysines: **hide sticks, lys**
Select Lys48+Lys63: **select lys48+63, 1UBQ and (resid 48,63) and not (name c+o+n)**
Show Lys48+Lys63: **show sticks, lys48+63**

Where in the ubiquitin structure are the two important Lys residues located?

The structure of diubiquitin

There is a reported crystal structure of Lys63-linked Diubiquitin in the PDB. Go to the PDB website and look up PDB ID 2JF5. In the experimental details section, look at the R-factors and the resolution. Would you call it a high-quality structure?

Nevertheless, let's look at this structure in PyMOL. Bring it up in your viewer:

Load structure:	**fetch 2JF5**
Hide lines:	**hide everything, 2JF5**
Show cartoon:	**show cartoon, 2JF5**
Align structures:	**align (2JF5 and chain A), 1UBQ**
Re-center view:	**orient 2JF5**

You should now see three ubiquitin chains. Two will be right on top of each other, one will be off to the side. Let's ignore 1UBQ for now, even though it would be interesting to look at conformational changes as a result of the diubiquitin linkage. Instead, let's look at the linkage between the ubiquitin molecules of 2JF5.

Hide 1UBQ:	**hide everything, 1UBQ**
Select residues in linkage:	**select link, 2JF5 and ((resid 63 and chain A) or (resid 76 and chain B))** (NOTE: make sure to get the parentheses right)
Show linkage:	**show sticks, link**

What type of chemical bond is present? How could it get formed?

Now, let's see if the two ubiquitin molecules in di-ubiquitin share a lot of surface area, i.e. if they seem to form a stable complex via protein-protein interactions. We can do that by changing to surface representation:

Show surface:	**show surface, 2JF5**

Does it look like the two ubiquitin chains would interact in the absence of the covalent linkage?

Last, let's make a nice figure of diubiquitin. Let's switch back to cartoon representation.

hide surface, 2JF5
show cartoon, 2JF5 (if necessary)
show sticks, link
color yelloworange, 2JF5 and elem C

You can change the color to your favorite color and rotate the view or zoom as you wish. You can also highlight other residues as you wish (following the procedure used for the Lys residues) and color the two chains individually by adding additional modifiers, e.g.:

color forest, 2JF5 and elem C and chain A

Once you are happy with the view, you can generate a nice high resolution figure by ray-tracing:

ray-trace: **ray 640,480** (this will generate a figure of 640x480 pixels)
save as png: **png path/diubiquitin.png**

You can also just save the current view as a png, but ray-tracing makes for a much nicer figure.

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