

PyMol Tutorials - Supplemental

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For further information:

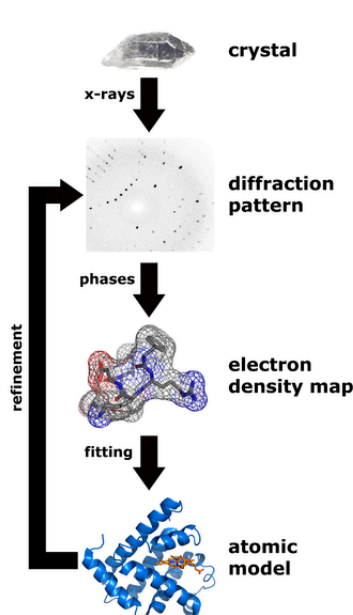
http://pymolwiki.org/index.php/Display_CCP4_Maps

Desktop Molecular Graphics

PyMol 3 – Electron Density Map

✓ READ:

Electron density is the measure of the probability of an electron being present at a specific location. In protein crystallography, an electron density map averaging all the molecules within the crystal allows a crystallographer to build a model of the molecule.



Workflow for solving the structure of a molecule by X-ray crystallography. (source: wikipedia)

Calculation of the electron density map¹: The data collected from a diffraction experiment is a reciprocal space representation of the crystal lattice. The position of each diffraction 'spot' is governed by the size and shape of the unit cell, and the inherent symmetry within the crystal.

The intensity of each diffraction 'spot' is recorded, and this intensity is proportional to the square of the structure factor amplitude. The structure factor is a complex number containing information relating to both the amplitude and phase of a wave and is a mathematical description of how a material scatters incident radiation.

In order to obtain an interpretable electron density map, both amplitude and phase must be known (an electron density map allows a crystallographer to build a starting model of the molecule). The phase cannot be directly recorded during a diffraction experiment: this is known as the phase problem.

Initial phase estimates can be obtained in a variety of ways including molecular replacement and heavy atom derivatives.

The final atomic model is deposited after a series of refinements. Some authors choose to deposit the electron density map or a structure factor file that can be used to calculate an adequate electron density map.

¹ http://en.wikipedia.org/wiki/X-ray_crystallography

The Protein Data Bank at the is the primary source of atomic models. However it now links to the Electron Density Server (EDS)² when a map is available.

“The Uppsala Electron Density Server (EDS; <http://eds.bmc.uu.se/>) is a web-based facility that provides access to electron-density maps and statistics concerning the fit of crystal structures and their maps.”

1 *PyMol3* - Exercise A: Download an electron density map

We will use the now familiar 2BIW entry, biological unit 2 which was the basis of multiple exercises in previous sections. The file `2biw.pdb2` should be on your desktop or you can download it again from the web browser link below.

The electron density map can be obtained directly from the EDS server or via a link to the EDS server from the PDB page. In both cases the user will land on the page on the EDS server.

1.1 Go to Electron Density Server page



TASK

- **Open a web browser** such as Safari or Firefox.
- Point your web browser to **www.rcsb.org**
- In the Search box **type** the following ID: **2biw**
- Then **click Search** button

If the PDB entry has an EDS file, the relevant link will be provided within the “Experimental Details” panel on the bottom right of the entry page.



TASK

At the bottom right of the entry page **click on the EDS link** (shown by arrow)

Experimental Details

Method: X-RAY DIFFRACTION

Experimental Data: [EDS]

Resolution [Å]: 2.39

R-Value: 0.18

R-Free: 0.224

Space Group: P 2₁ 2₁ 2₁

Unit Cell:

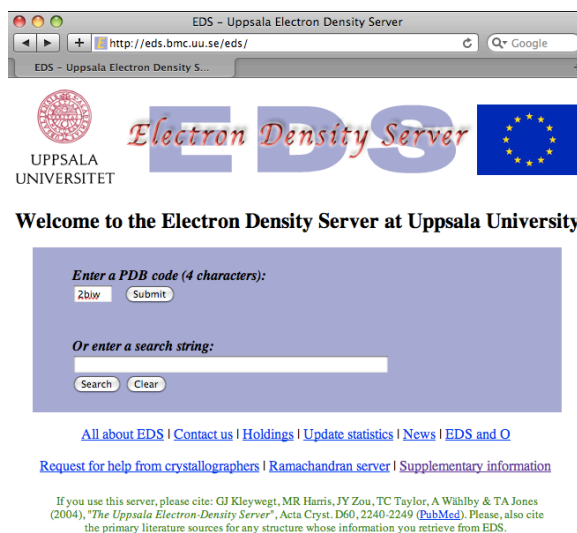
Length [Å]	Angles [°]
a = 119.05	α = 90.00
b = 125.28	β = 90.00
c = 203.09	γ = 90.00

² The Uppsala Electron-Density Server. G. J. Kleywegt, M. R. Harris, J. Zou, T. C. Taylor, A. Wahlby and T. A. Jones. *Acta Cryst.* (2004). D60, 2240-2249 doi:10.1107/S0907444904013253

✓ INFO

Alternate method: go to the EDS server and enter 2biw in the PDB code box and click submit.

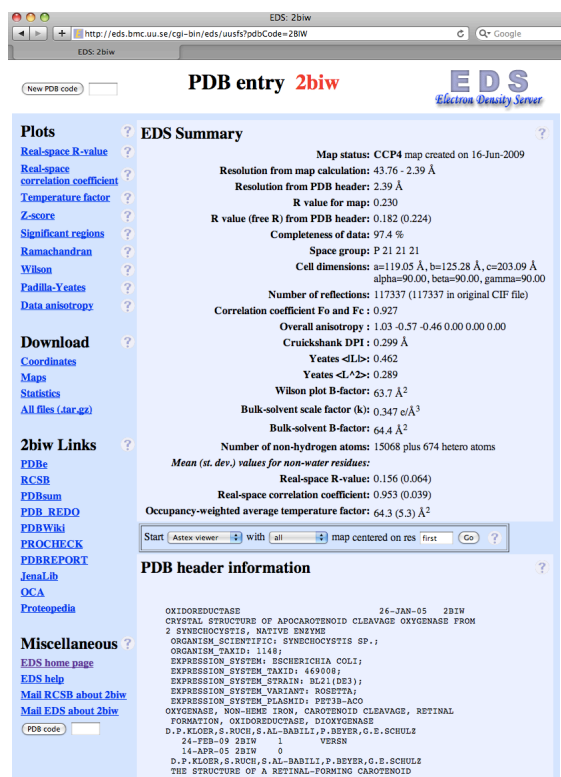
Regardless of the method, the end page will be the same.



✓ INFO

The EDS summary page lists most parameters related to crystallography.

The bottom part of the page echoes the header portion of the actual PDB data file.



1.2 Download the electron density map in CCP4 format

Just like everything else for computer files, there are various formats available. CCP4³ is a format Pymol understands.

✓ TASK

- Under the **Download** tab **Click on Maps**

- Select CCP4** as the map format
- Keep Type** the same

Electron-density map generation for 2biw

Map format: ☒ O ☐ CCP4 ☐ CNS ☐ ESD
 Type: 2mFo-DFc

(Note: this may take a few seconds, or many minutes, depending on the size of your map.)

- Press “Generate map” button**

Electron-density map generation for 2biw
 Generating map....
 This may take a few seconds, or many minutes, depending on the size of your map.
Here is your zipped map: 2biw.ccp4.gz

- Download** the proposed file
- Rename the uncompressed** file: 2biw.map.ccp4

Note: the renaming will help in the Names panel within PyMol.

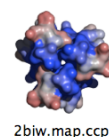
2 PyMol3 - Exercise B: Display electron density map

(Assumes you completed previous exercise and downloaded map)

2.1 Open PyMol and Map

✓ TASK

If the file has a PyMol icon, simply **double-click the icon** to open a new PyMol session. Or simply launch PyMol and use the File > Open menu.



The top section of PyMol will echo information about the opened file:

```
ObjectMapCCP4: Map Size 160 x 145 x 155
ObjectMapCCP4: Normalizing with mean = -0.008028 and stdev = 0.211077.
ObjectMap: Map read. Range: -2.930 to 8.486
Crystal: Unit Cell      119.050  125.275  203.087
Crystal: Alpha Beta Gamma  90.000  90.000  90.000
Crystal: RealToFrac Matrix
```

³ The Collaborative Computational Project Number 4 in protein crystallography or (CCP4) was set up in 1979 in the United Kingdom to support collaboration between researchers working in software development & assemble a comprehensive collection of software for structural biology. 1979. http://en.wikipedia.org/wiki/Collaborative_Computational_Project_Number_4

```
Crystal: 0.0084 -0.0000 -0.0000
Crystal: 0.0000 0.0080 -0.0000
Crystal: 0.0000 0.0000 0.0049
Crystal: FracToReal Matrix
Crystal: 119.0500 0.0000 0.0000
Crystal: 0.0000 125.2750 0.0000
Crystal: 0.0000 0.0000 203.0875
Crystal: Unit Cell Volume 3028845.
ExecutiveLoad: "/Users/dmc/Desktop/2biw.map.ccp4" loaded as "2biw.map", through state 1.
```

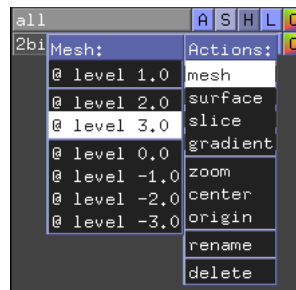


Do not panic if your screen still appears black at this point! This is normal.

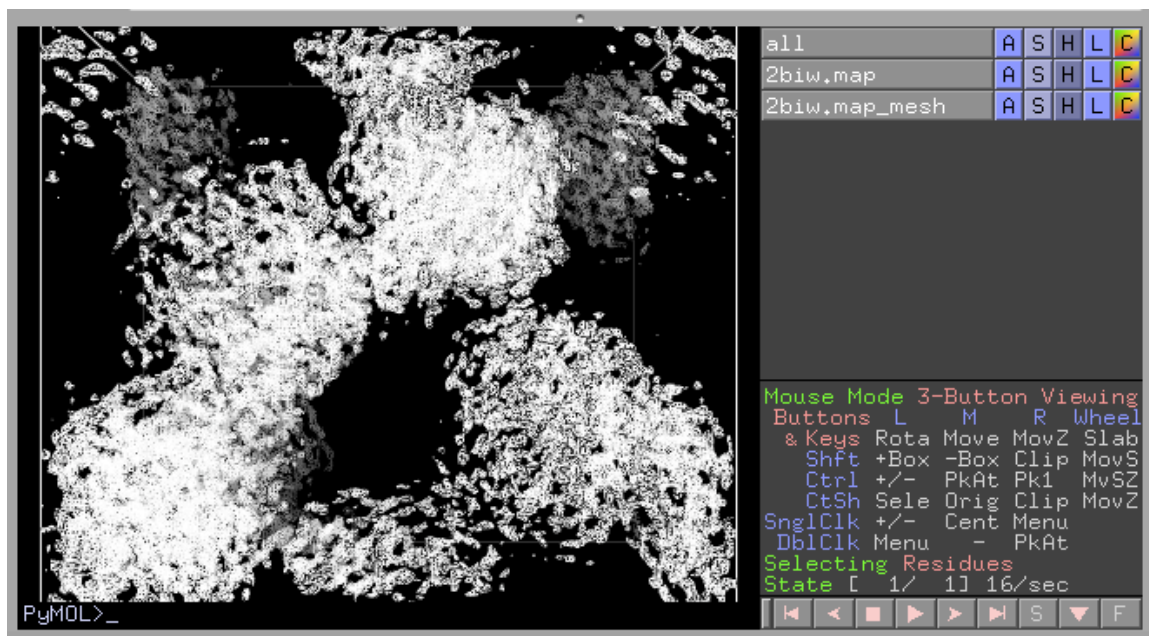
In the Names panel **click** on **2biw.map** to let the volume of the map appear as an empty cube.

Then:

Click on Action (A) and select **mesh @ level 3.0** to show the corresponding electron density.



This will create a new entry in the Names Panel called 2biw.map.mesh



The electron density map for the complete section of space is presented at the level 3.0. Of course that is too much information and we will reduce the section of the map that is shown and play with the density level as well.

Note: we selected the “mesh” option within the Action menu. There is also a “surface” option which could be useful in some cases, perhaps with added transparency.

2.2 Change electron density levels

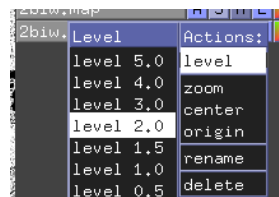
Typically, contour level values are reported in the absolute value of electrons/Å³.



TASK

Click Action button for name 2biw.map.mesh and select **level 2.0**

This will change the displayed density in the viewer.



You can check the effect for other values, and then come back to a value of 2.0

3 *PyMol3 - Exercise C: Add PDB and reduce displayed area*

File 2biw.pdb2 should be on you desktop and the electron density map should be displayed at level 2.0 from the previous section.

Open the PDB data within PyMol with the line commands:



TASK

```
cd ~/desktop
load 2biw.pdb2
```

When the PDB file is loaded, PyMol automatically zooms on the location of the atom coordinates. The familiar bonds between atoms are masked by the electron density map. The many other sections of the electron density map representing crystallographic symmetry can be later removed for clarity.

3.1 Display electron density for the complete protein

The following commands select which region of the map to display (variable name *site* chosen for the complete protein) and the next command creates a new map around the selected site:



TASK

```
select site, 2biw.pdb2
isomesh map, 2biw.map, 2.0, site, carve=1.6
```

This will create a local map under the name “map” within the Names Panel.

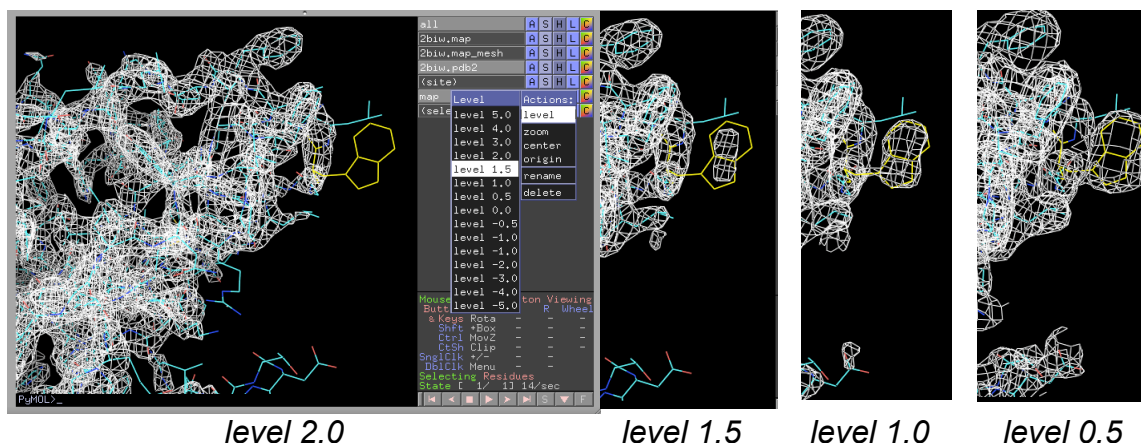
In the Names Panel **click on 2biw.map.mesh** to hide the general mesh map. This will reveal the local map around the single protein.

3.2 Illustrate electron density resolution via map levels

✓ TASK

Rotate the structure to better see Tryptophane 121

Change map level to 1.5, 1.0 and then to 0.5 (map > A > level > 1.0) to illustrate the increase of electron density map drawn at this location. It is due to the lower resolution of the map at this site: most W121 atoms would have a higher temperature factor within the PDB file.



Note that ASP 331 at the bottom of the page follows the same pattern of electron density map plots as the levels vary.

4 PyMol3 - Exercise D: display a local area only

Starting point: previous exercise showing map at level 0.5.

We will select a local segment which is in alpha-helix configuration and display the electron density of this local area.

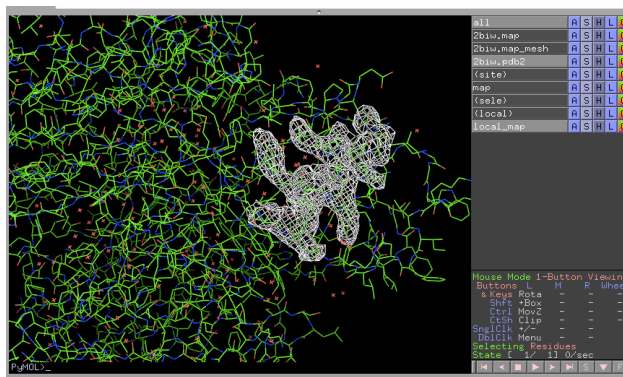
✓ TASK

select local, resi 95-105
isomesh local_map, 2biw.map, 1.0, local, carve=1.6

Note: the words “local” and “local_map” are created by the user and are not PyMol commands reserved words.

On the Names Panel
Click on “map” to
remove it from view.

Only the wireframe of
the protein should
remain and the local
electron density plot we
just created.



✓ **TASK** - With the mouse do the following:

- Hide the protein wireframe:
- Show the local helix in wireframe:
- **Zoom** on the local area with the mouse.

2biw.pdb2 > H > everything
local > S > sticks

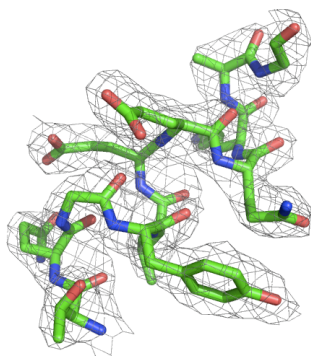
Using line commands:

```
center local      # change center of rotation to local area
color gray50, local_map # set color to 50% gray
bg_color white    # change background to white
set mesh_width, 0.5 # makes meshes thinner, for raytrace
```

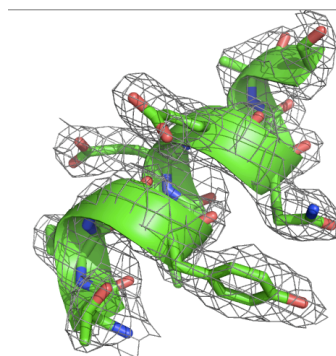
The following are optional settings: remove # to activate.

```
# set ray_trace_fog, 0 # turn off raytrace fog;
# set depth_cue, 0    # turn off depth cueing
# set ray_shadows, off # turn off ray-tracing shadows
```

Press the “ray” button or **type ray** within the line-command are to finish.



Raytraced image from above settings



with additional cartoon & thicker mesh width (1.0)

