

PSD 2016 -- Xray Lab

- 1) CCP4 lab
- 2) Phenix lab
- 3) Coot lab

===== Install. =====

Download, install CCP4. www.ccp4.ac.uk/

Download, install Coot. biop.ox.ac.uk/coot/

Download, install Phenix. www.phenix-online.org

Download images from data collection:

<http://www.biointro.rpi.edu/bystrc/courses/bcbp4870/ATDATA.zip>

(linked to [Full Xray data set](#) on the course web page)

Download files linked to:

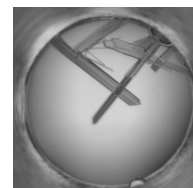
[COOT tutorial PDB file](#)

[COOT tutorial MTZ file](#)

[PDB for Phenix](#)

[MTZ for Phenix](#)

[SEQ for Phenix](#)



Crystals of a designed GFP mutant with improved folding properties, GFP-AT.

===== "Collect data" =====

The data set you are given was collected from crystals of a variant of green fluorescent protein (GFP) called GFP-AT. GFP-AT folds more efficiently than GFP. The reason for the slow folding of wild-type GFP have to do with the very slow rotation of a peptide bond at Proline 89 in the wild-type sequence. When the Proline is mutated away and replaced with a loop of three residues, the peptide bond rotation no longer happens, and the protein folds faster. To see what the loop addition did to the structure, we need to solve the structure.

GFP-AT was crystallized at pH 7.5 using 20% polyethylene glycol mw 8000. The crystals were frozen in liquid nitrogen on nylon loops and transported in a dewar to Brookhaven National Labs Synchrotron Light Source for data collection. Data was collected using a CCD, 175mm from the crystal. The beam position was $x=80.896\text{mm}$, $y=80.896\text{mm}$. The wavelength of the X-rays was 0.9790 \AA . 1° oscillations were collected from -10° to $+190^\circ$ in the phi angle (laboratory vertical axis), but the last 15 frames appear to be weak, indicating that the crystal was getting damaged. .

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1) =====

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Reduce the data.

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Open CCP4

Save your session periodically, in case something happens. You can start from a saved session.

Choose module: Data Reduction. Data processing using iMosFlm. Start iMosFlm.

In iMosFlm window:

Settings/Environment variables... Choose a Working directory. iMosFlm will send files here.

Session/Add images... Files of type: *Numbered files*. Select x3a-c-sw1.0001 through x3a-c-sw1.0050.

You should see images appear. You can flip through these using the blue arrows. Note how the spots move. Some spots show up across several frames. This is a sign of high mosaicity.

Select Settings/Experiment settings. Look but don't touch. They should be correctly set already based on information in the image headers.

Select Indexing. The program tries increasing the box size (in degrees) until the whole spot fits in the box. The size of the spot is the mosaicity. How mosaic is the crystal?

Select cell refinement. Press the "a" button to automatically select images. Process. Fix the cell dimensions. Process again. Scroll through the spot images to see the average spot in each image, its integration box and its background box.

Select Settings/Processing options. Look but don't touch.

Select Integration. Process. Scroll through average spot images. Did the spots get bigger towards the end of data collection? Scroll through block spot images. Are the spot shapes different on different regions of the images? Why?

Select QuickSymm on menu bar to run Pointless. Select Show Full Logfile. What kind of symmetry does this data have? Does the data pass the twinning test? Does the data have systematic absences? Does the data suggest we re-integrate the data? OK.

Repeat from the beginning by selecting **start new session**. Don't save. Session/Add images. Re-reading the image headers resets the parameters.

Select Indexing. This time set the space group to P222. Index. Look at image window. Click on Show Predictions (boxes). Do the boxes surround the spots? Zoom in on a spot until you see the numbers. How flat is the background?

Select Cell Refinement. Are there warnings? Read them.

Select Integration. Process.

Select QuickScale. (It is not "quick".) Results will open in a web browser. Look at Axial reflections in a^* , b^* , and c^* . Are there systematic absences? What is the space group?

Save this page as a **PDF file**. Upload it to the course upload page as **Part 1**.

NOTE: Questions posed here are for guidance only. I don't expect you to send answers.

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2) ===== Solve the structure using molecular replacement. =====

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Download **CLA_0.pdb**, **CLA.seq**, and **CLA_v2.mtz** from the course web page if you have not already done that.

Open **Phenix**

Start **Phaser-MR** (normal interface)

Under the **Configure** tab, **input and general options**, browse for the data file **CLA_v2.mtz**

The unit cell and space group parameters show up automatically.

Set **high resolution** and **high resolution for refinement** both to **2.7**

Choose an output directory and a title. Put your name in the title.

Select **Ensembles** tab.

Add file: **CLA_0.pdb** (This is your search model)

Select it. Click "**Change variance**" and set **RMSD** to **1.5**.

Set **Model ID** to 1.

Select **Composition** tab.

Set **number of copies** to 3 (We think there are 3 molecules in the asu)

Set **Sequence file** to **CLA.seq**

Select **Search Procedure** tab.

Select model 1. Set **Copies** to 3.

Hit **Run / Run Now**.

View log file (ask questions!).

Export the log file as PDF and upload it as **Part 2** on the Homework server page.

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3) =====

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Manual refinement in Coot.

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Follow the instructions in the Coot Tutorial, linked to the course web page. Go as far as modeling the "3 blobs".

<https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/web/tutorial/tutorial.pdf>

Download the files linked to

COOT tutorial PDB file

and

COOT tutorial MTZ file

from the course web page.

Follow the instructions in the tutorial. Do up to the end of section **4 Blobology**, or get as far as you can through the tutorial as time allows.

Save the coordinates (protein and any additional ligands or waters that you added) and concatenate them together in a text editor. Output the file as plain text with the **".pdb"** file tag. Upload this file on the homework server as **Part 3**.