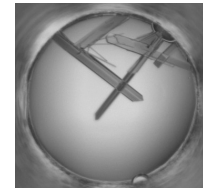


PSD 2011 -- Homework 6

Due Dec 5.

Upload your homework in 4 parts to
<http://www.bioinfo.rpi.edu/bystrc/courses/bcbp4870/homework.html>



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

===== Solve a crystal structure =====

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.

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

Download, install CCP4. <http://www.ccp4.ac.uk/>

Download, install Coot. <http://biop.ox.ac.uk/coot/>

Download images from data collection:
<http://www.bioinfo.rpi.edu/bystrc/courses/bcbp4870/ATdata.zip>

Download molecular replacement model:
http://www.bioinfo.rpi.edu/bystrc/courses/bcbp4870/AT_model_clean2.pdb

Download pre-calculated reduced data:
http://www.bioinfo.rpi.edu/bystrc/courses/bcbp4870/x3a-c-180-output_sfall2.mtz

===== Reduce the data. =====

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. 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.

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

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

Download the molecular replacement model.

"Molrep model"

http://www.bioinfo.rpi.edu/bystrc/courses/bcbp4870/AT_model_clean2.pdb

Ignore the reduced data from the first part and download reduced data from the web site.

Linked to "Molrep MTZ file"

http://www.bioinfo.rpi.edu/bystrc/courses/bcbp4870/x3a-c-180-output_sfall2.mtz

Choose module: Molecular replacment. Select Run Molrep. Use the data (MTZ file) and model (PDB file) that you downloaded. Name the output PDB file xyz.pdb (or any name you like.) Use the defaults: rotation & translation function. Fp = F_AT_1.

Find the log file (ends in .log.html). Open it in a web browser, click on "Show full logfile" and print it to a PDF file. Upload the PDF file to the course upload page.

===== Calculate a map using MR phases. =====

Choose module: Map & Mask utilities. Select Run FFT - Create map. Choose nF1-mF2, and in "define map" set n=2 and m=1.

Use F1 = F_AT_1, F2= Fcalc (calculated from PDB coordinates), PHI (phase) = PHIcon (calculated from PDB coordinates). SIGMA=SIGF_AT_1, WEIGHT=unassigned, SIGMA (F2) =unassigned. PDB file = xyz.pdb (if that's what you named it). Map out = xyz.map. Output in CCP4 format to cover all atoms in PDB file.

Find the log file (end in fft.log.html). Open it in a web browser, click on "Show full logfile" and print it to a PDF file. Upload the PDF file to the course upload page.

Close CCP4.

===== View the map in Coot. =====

From the course web site:

Download electron density map linked to "**Fobs map**" on the course web site.

Download coordinates linked to "**Unrefined coordinates**."

Display the coordinates (Open model...) and map (Open map...) in Coot. Find locations in the structure that are **well modeled**, and other locations that are **not well modeled**.

Save images. (screen capture?) Read images into a powerpoint or word file. Add a

caption for each image describing why you think the structure is well modeled or not well modeled. What would you do to fix the model?

Save the images and captions together as PDF and upload it to the course upload page as the last item on that list.

===== extra credit =====

For extra credit, use the **real-space refinement tools** to improve the model. Save the coordinates in PDB format* and use them in CCP4 (FFT tool) to calculate a new Fobs map. Make an image of the refined region before and after refinement.

*You may have to add header information to your PDB file. If so, copy it from the "Unrefined coordinates" PDB file.