

Bioc585 2008 Exercise 6 – Structure Factors from the PDB
Homework due Tuesday, May 6

We now have assembled all of the tools needed to pull structures and structure factor amplitudes from the Protein Data Bank for viewing models and electron density. For this exercise, we will perform the necessary steps to accomplish this, and explore structural aspects of calmodulin complexes.

1) Files for calmodulin bound to an anticancer drug. A drug designed to interfere with tubulin polymerization turns out to bind with good affinity to calmodulin. This work is described in: Horvath et al. (2005), *J. Biol. Chem.* 280:8266-8274. The PDB file is 1XA5.

1. Create a new directory for today's exercise such as ex6.
2. Go to the PDB (www.rcsb.org/pdb) and type 1xa5 into the search window.
3. Select **Download Files** from the menu on the left and download the pdb file (PDB text). The default location for this file is Desktop/Downloads with filename 1XA5.pdb.
4. Download the **Structure Factor Text** file. This will likely pop up as text in your browser window, which you can ignore by closing the program. The default filename in your Downloads directory should be 1xa5-sf.cif.
5. Go back to the home PDB page, download the PDB file for 1cdl and move it into the ex6 directory.

2) Creating a MTZ file.

1. Start CCP4i and create a new project (e.g. 1xa5). Be sure to select your new project for this session, and Apply&Exit.
2. Select **Reflection Data Utilities** and **Convert to MTZ & Standardize** to get the import menu. Fill in all values as follows.
3. Import reflection file in **mmCIF** format.
4. **Keep existing FreeR data.**
5. In: **1xa5-sf.cif** (or whatever you called your structure factor file).
6. Out: **1xa5-sf.mtz** (or default).
7. Choose names for crystal and dataset (e.g. 1xa5).
8. Fill in the space group and cell constants. These values can be found in the PDB file open with gedit).
9. Run the job.

3) Run Refmac. This will need to be done twice in order to create a dictionary for the non-standard portions of the complex.

1. Run the job once using typical Refmac values. It will fail due to the missing library file for the drug (should happen in 1-2 minutes). Open the log file to confirm this.
2. Run the job again but this time reading in the new **Library** file put out in the first Refmac job. It will be called something like **ex2_2_lib.cif**. The job should be successful this time.

4) Examine the structure and electron density with Coot. Look at the quality of the electron density for the inhibitor. Is it convincing? How is the ligand held in place?

5) Examine the electron density for the “central helix”. Examine residues 76-81, for example, that were dynamic in the NMR experiment.

6) Read in 1CDL.pdb. This is the file for calmodulin bound to a peptide from smooth muscle myosin light chain kinase (Meador et al (1992), *Science* 257:1251).

1. Select **SSM Superpose** (in the Calculate menu).
2. Superpose 1cdl chain A onto 1xa5.
3. Display both files as **C-alfas + Ligands**. Do the ligands occupy similar spaces? Are the protein conformations the same?
4. Superpose 1cdl chain B onto chain A (select “Move copy of moving structure”). Turn off the display for 1xa5 so that it is easier to see 1cdl chains A and B. Do the two chains have the same conformation?

Homework 6 – 10 points

1. For structure 1XA5, include a snapshot of the geometry vs. refinement cycle graph from Refmac.
2. For structure 1XA5, describe the linking domain (“central helix”) in the model and the electron density for this region. Include a snapshot to illustrate your conclusions regarding geometry and structure quality.
3. For structure 1XA5, calculate an “omit” map for either the linking domain or the inhibitor, and include a snapshot of the result. Is the omitted portion well represented?
4. On comparing 1XA5 and 1CDL, do the bound helix and the bound inhibitor occupy similar places in calmodulin? Do they induce similar calmodulin conformations? Include a snapshot.
5. How many calmodulin molecules are in the asymmetric unit of 1CDL? How well do the A and B subunits compare?