Bioc585 2006 Exercise 4 – MAD Phased Model
Homework Due April 22

This exercise makes use of MAD phasing for an 85 amino acid protein called CusF, from the McEvoy lab. The protein was expressed with Se-Met rather than methionine, leading to three selenium sites. The wild type and derivatized proteins crystallized and diffracted in-house to 1.7 Å resolution. These crystals were flash frozen and shipped to the Brookhaven National Laboratories synchrotron, where a staff scientist measured MAD data from a Se-Met crystal (2.0 Å), and Native data from a wild type crystal (1.5 Å). These data were of sufficient quality to allow a strong first model to be built using automated chain tracing software. We will look at maps and models along this path.

Space group: $P2_12_12_1$
Unit Cell (Se-Met): a = 39.05 Å, b = 40.43 Å, c = 44.03 Å, $\alpha = \beta = \gamma = 90$
Unit Cell (wild-type): a = 39.30 Å, b = 39.33 Å, c = 43.69 Å, $\alpha = \beta = \gamma = 90$

1. Copy the sub-directory MAD and all the files inside to your home directory. Several PDB and MTZ files can be found therein.

2. MAD data were measured at three wavelengths:

Peak Absorbance: λ = 0.9781 Å
Inflection: λ = 0.9788 Å
Remote: λ = 0.9600 Å

Numerous peaks were found in the Patterson maps. The expected number of peaks in the asymmetric unit due to interatomic vectors, excluding the self-self peaks at the origin, is N^2-N = 6. In the unit cell, this number is 24 (6x4).

The resulting positions, occupancies and temperature factors can be found in se.pdb. Open this file in gedit (Accessories > text editor) and look at the values.

3. Three selenium positions were identified in the Patterson and their positions refined using the automated program Solve. This lead to phases with Figure of Merit (FOM) = 0.53 (better at lower resolution).

The initial electron density map can be produced with solve.mtz. Start Coot, read in se.pdb. Open solve.mtz with the "Open MTZ ..." menu (under "File"). This must be done because the labels for the data do not match the expected labels in "Auto Open...". The map we want is the Fourier transform using FP and PHIB (experimental amplitudes and phases), weight (an error weighting parameter). FP and PHIB are the defaults, but "use weight?" must be selected, then click "OK". Move around this map and see if protein can be identified (cntrl-left-mouse-button to translate). Close Coot when finished or close all files ("File menu").

4. Density modification and partial automated model building was performed by the program Resolve. This program finds the molecular boundary, flattens peaks in the solvent (solvent flattening) and searches for contiguous electron density that matches the known sequence. Where successful, model is built.

Start Coot and read in resolve.pdb and resolve.mtz, as in section 3. Note the largely correct interpretations of the map, and the improvement in the map quality. Close Coot when finished or close all files ("File menu").

5. The program Arp/Warp was used to automatically build a better model and extend the phases to 1.5 Å for the wild type crystals. Some sequence was identified correctly, some not and filled in as glycines.

Start Coot and read in warpNtrace.pdb and warpNtrace.pdb. Here, weighted 2Fo-Fc amplitudes and phases are available, so choose this in the MTZ window (called 2FOFCWT and PH2FOFCWT). Do no choose a weight since it is already included. Move about the model and map and observe the quality improvement. Center on Gly 13 T and use "Simple Mutate" to build the correct residue (in the "Model/Fit/Refine" menu, under "Calculate"). If time permits, build in as much of the glycine chain as possible and refine the resulting model with "Refmac" in CCP4.

6. A nearly complete model with phases can be found in cusf.mtz and cusf.pdb. Check your model from section 5 with this model.

Bioc585 2006 Exercise 3 – MAD Phased Model
Homework Questions: 10 points

The following questions refer to computer exercise 3.

(1) Starting with the warpNtrace map and model, build in side chains for Gly 11 T, Gly 12 T and Gly 13 T. Refine this model with REFMAC and examine the new model and map. If this model looks good (matches the density well), copy and paste an image into your answer. If not, pick a different residue and try again (hint – watch Gly 12 T carefully). Also copy and paste an image of your refinement log graphs for R-factor and geometry (the last graph, Rfactor analysis, stats vs cycle).

(2) View the density for these three residues in the beginning experimental map (using solve.mtz). How do they compare?

(3) Examine the positions for the selenium atoms (from crystal 1, see introduction) and compare with the warpNtrace model (based on crystal 2). How well do they align with the methionine sulfurs? Which methionine is associated with which selenium? Suggest an explanation for any deviations.

(4) Are the selenium atoms all attached to the same molecule in the unit cell? Please explain your conclusion.