

Recent MAD structures determined at CHESS

Daniel J. Thiel and Richard L. Walter

Station F2 continues to grow in popularity as the beamline for carrying out multiwavelength anomalous diffraction (MAD) experiments at CHESS. Over the last 14 months almost 50 % of the scheduled beamtime has been delegated to protein crystallographers requesting to perform MAD phasing experiments. Based on the volume of interest expressed by our users, we foresee even greater allocation of beamtime to MAD experiments in the future.

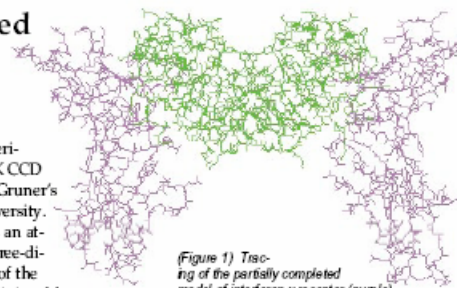
This station receives radiation from the F-line wiggler which is doubly-focused using both a sagittally-bent monochromator and a rhodium-coated bent mirror. Compared to stations A1 and F1, the flux at this station is reduced by roughly a factor of 5 at the comparable x-ray energy. However, this loss, primarily due to the more narrow energy bandpass of the monochromator, is compensated by versatility, namely, tunability of x-ray energy from 7 to 25 keV. In addition, the energy resolution of the monochromator is sufficiently low so as to provide a monochromatized beam suitable for XAFS, optimized anomalous scattering, and MAD experiments.

The most recently published MAD work from CHESS include experiments from the labs of Steitz (Yale University) and Aggarwal (Columbia University). The Steitz lab solved the structures of both the core fragment of *lac* repressor [1] and the phage T4 gene 32 DNA binding protein [2], and the Aggarwal group produced the structure of *bamHI* [3]. These structures were determined from data collected using the MacCHESS Fuji image plate systems.

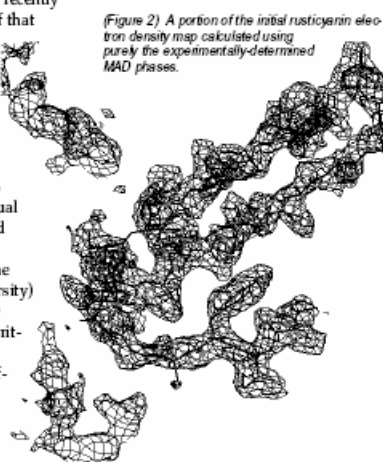
More recently, the acquisition of CCD detectors by MacCHESS has had a profound effect on the collection of MAD data at station F2. In addition to making the entire process of collecting the MAD data more efficient, the CCD detectors have proven capable of acquiring diffraction data of sufficiently high quality to solve challenging MAD phasing experiments as shown by the early results of the two initial MAD/CCD measurements which were conducted by the research group of Steve Ealick.

Both of these experiments utilized the 1K CCD detector built in Sol Gruner's lab at Princeton University. The first experiment, an attempt to solve the three-dimensional structure of the 155 amino acid containing, blue copper protein rusticyanin, has recently resulted in the determination of that structure to 2.2 Å resolution. A three-wavelength data set was collected from unfrozen, monoclinic crystals of the protein around the copper K edge. In the end, data from four crystals were combined to make a data set which was 92% complete to 2.2 Å. The individual crystal data were processed and phased separately using the MADSYS programs from Wayne Hendrickson (Columbia University) and elements of a second MAD phasing program, MADPRB, written and provided by Alan Friedman (Yale University). After MAD processing, the data were combined and electron density was calculated. After only standard solvent flattening, these maps were of high enough quality (see Figure 2) to unambiguously align the amino acid sequence and trace the entire chain of the molecule without breaks from N to C terminus, with the exception of the five N-terminal residues. The model is currently being refined.

In addition, we have used the MAD technique to solve the structure of the extracellular portion of the interferon- γ receptor complexed with interferon- γ . Much of the success of the project is credited to Marie LeDu, a postdoc in Steve Ealick's lab who recently accepted a staff position at CE Saclay in Paris. This complex contains nearly 700 amino acids and has a molecular weight of 100 kD. The data were collected using frozen crystals (space group C2) containing 8 seleno-methionine residues within the interferon- γ portion of the complex. The MAD data (75% complete to 3.8 Å) was brought to a common scale by anisotropic



(Figure 1) Tracing of the partially completed model of interferon- γ receptor (purple) complexed with interferon- γ (green).



(Figure 2) A portion of the initial rusticyanin electron density map calculated using purely the experimentally-determined MAD phases.

scaling to a 2.7 Å native data set collected using the 2K CCD detector. The phasing was treated as a conventional heavy-atom problem with the inclusion of anomalous scattering. In Figure 2, a tracing of our present model is shown. Phase improvement is still underway.

Following these two initial experiments, nine outside users have collected MAD/CCD data at station F2. Structural results from several of these groups are anticipated.

[1] A. M. Friedman, T. O. Fischmann, and T. A. Steitz. Crystal structure of *lac* repressor core tetramer and its implications for DNA looping. *Science*, 1995, (in press).

[2] Y. Shamo, A. M. Friedman, M. R. Parsons, W. H. Konigsberg, and T. A. Steitz. Crystal structure of a replication fork single-stranded DNA binding protein (T4 gp32) complexed to DNA. *Nature*, 1995, (in press).

[3] M. Newman, T. Strzelecka, L. F. Damer, I. Schildkraut, and A. K. Aggarwal. Structure of restriction endonuclease *bamHI* and its relationship to *ecoRI*. *Nature* 368, 660-664 (1994).