Recent MAD structures determined at CHESS
Daniel J. Thié and Richard L. Walter

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

This station receives radiation from the Cu Kα1, which is double-focused using both a sagitally-mounted 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 bandwidth of the monochromator, is compensated by versatility, namely, tunability of x-ray energy from 7 to 28 keV. In addition, the energy resolution of the monochromator is sufficiently low so as to provide a monochromatized beam suitable for MAD, optimized anomalous scattering, and MAD experiments.

The most recently published MAD work from CHESS include experiments from the labs of Stueck (Yale University) and Aggarwal (Columbia University). The Stueck lab solved the structures of both the core fragment of the repressor [1] and the phage T4 gene 32 DNA binding protein [2], and the Aggarwal group produced the structure of barnHi [3].

These structures were determined from data collected using the MacCHESS fast imaging 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 Balkin.

Both of these experiments utilized the 64 CCD detector built at Sol Gurney'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 rubredoxin, has recently resulted in the determination of that structure to 2.2 Å resolution. A three-wavelength data set was collected from 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 MADDSYS programs from Wayne Hendrickson (Columbia University) and elements of a second MAD phasing program, MADPHB, 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 complex with interferon-γ. Much of the success of the project is credited to Marie LeDuy, a postdoc in Steve Balkin's lab who recently accepted a staff position at GEM Sciences in Paris. This complex comprises nearly 200 amino acids and has a molecular weight of 100 Kd. The data were collected from five crystals (space group C2) containing 8 selenium-methionine mutiplies within the interferon-γ portion of the complex. The MAD data (56% complete to 2.8 Å) was brought to a common scale by anastomopic 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, many outside users have collected MAD/CCD data at station F2. Structural results from several of these groups are anticipated.