

MAD phasing on F2: crystallographic results and station developments

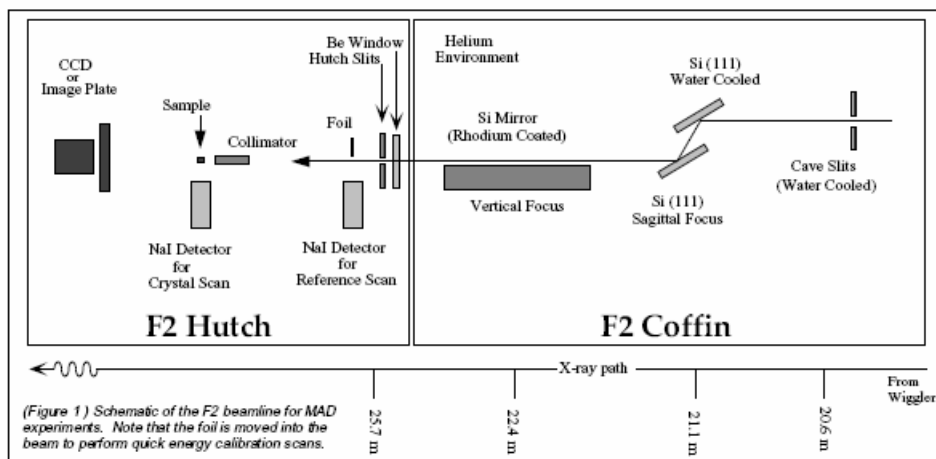
Dan Thiel, Park Doing, and Steve Ealick

Over the last few years, the crystallographic technique known as MAD, or multiwavelength anomalous diffraction, has emerged as one of the more powerful methodologies available to the macromolecular crystallographer.¹ Many structures have now been solved using this technique; however, along with the superior power of the method come extra instrumentation challenges facing the experimenter. At CHESS, the instrumentation for conducting MAD experiments is maturing to the point where outside users are now capable of carrying out MAD runs without relying on their own instrumental resources.

To carry out a MAD experiment, the researcher needs only one crystalline form of the sample - the use of heavy atom derivatives is eliminated. This crystal form must contain a low number of sites (typically between 3 to 10) of an elemental component with an atomic weight preferably in the range of $Z=20$ to $Z=40$ or $Z=60$ to $Z=92$. With the one crystalline form, the phase problem can be solved with essentially no ambiguity. This is done by tuning the incident x-ray beam to appropriate energies relative to the absorption edge of this particular atomic element and recording the diffraction data using these various x-ray energies. In such a man-

ner, the positions of these few atoms can first be solved forming a reference point for solving the entire structure.

Obviously, a tunable beamline with the usual high flux and low divergence is necessary to carry out such an experiment. At CHESS, such a facility exists at the F2 doubly-focused wiggler beamline. A 24 pole, 1.2 Tesla permanent magnet wiggler installed in a straight section of the 5.3 GeV Cornell Electron Storage Ring (CESR) emits 6.4 kW of radiation into a 4 mrad horizontal opening at 100 mA of positron current. Station F2 accepts 2 mrad of this radiation which is monochromatized by a



fixed offset, sagittally-focussing monochromator. A schematic of the station optics is shown in Figure 1. A rhodium coated Si mirror located downstream of the monochromator is used to focus the beam vertically and to suppress unwanted harmonics. This doubly focussed wiggler beam has a focal size of 3.6 mm x 0.32 mm in the hutch. At 13 keV, the flux is 10^{10} ph/s after a 0.3 mm collimator (at 100 mA).

Provisions have been built into the MAD setup which permit a rapid periodic check of the calibration of the monochromator to insure that the anomalous signal is being properly recorded. With one computer command, the experimenter rotates a reference foil into the beam at the upstream end of the hutch and initiates a quick scan of the monochromator through the edge energy. After the absorption spectrum is displayed on the terminal, the reference foil and the monochromator return to their starting positions. If the spectrum shows a shift in the calibration, the monochromator is set to the proper position. This process is typically carried out at the beginning and the end of each fill, consuming less than 1 minute of beamtime for each check. During a recent MAD experiment, a

series of reference scans done at the beginning of 4 fills over an 8 hour period showed an energy drift of less than one eV. While this degree of stability was often observed, variations of up to 4 eV have also been seen. Clearly, close monitoring of the calibration of the monochromator is still essential for a successful MAD experiment.

Using this facility, we have recently completed a MAD experiment in collaboration with Menachem Shoham of Case Western University studying the copper-containing protein rusticyanin. The data were collected using the CCD camera which has recently arrived at CHESS (see separate CCD article in this newsletter). Data processing is currently underway.

The results of some earlier MAD experiments demonstrate the effectiveness of the F2 beamline. In August 1992, Aneel Aggarwal's group from Columbia collected MAD data from the restriction endonuclease *bam*HI in which 5 methionine residues were replaced by seleno-methionines. A total of 5 crystals were shot at 3 wavelengths relative to the selenium absorption edge, corresponding to the edge, peak, and a remote wavelength above the edge.

The quality of the data was outstanding, with 97% of the intensities having I/σ greater than 3.0. This data resulted in a 1.95 Å structure which refined to an R-factor of 19%. From this structure, the researchers have noted that the protein resembles EcoRI despite the lack of sequence similarity.

Finally, Tom Steitz' group from Yale carried out MAD experiments on F2 resulting in the solution of two crystal structures. The core fragment of the E. Coli *lac* repressor was solved using the L_{23} edge of the anomalous scatterer Hg (12.284 keV) in a non-isomorphous crystal form. Also, a Se edge (12.658 keV) experiment resulted in the structure of the phage T4 gene 32 single-stranded DNA binding protein.

These results highlight the MAD phasing work that has occurred on F2. In the future, we expect to see two of CHESS' strengths exploited in the MAD work - using higher x-ray energies to probe the edges of other anomalous scatterers and carrying out the measurements with the more sensitive CCD camera rather than image plates. Additional exciting results are sure to follow.

*For a review see Hendrickson, W. A. Science 254 pp. 61-68, 1991.