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Status of the Practical Use of Dispersive X-rays for MAD Experiments in Biocrystallography : SMAD and DAFS

Preliminary SMAD and DAFS data were successfully collected from crystals of a cytochrome ($P2_12_12_1$ a=37.6Å b=43.8Å c=45.3Å) at the Fe K-edge (λ=1.74Å) and crystals of a cellulase derivative ($P2_12_12_1$ a=50.1Å b=63.5Å c=105.0Å) at the Hg L_{III}-edge (λ=1.01Å). The method employs a bent monochromator crystal to produce a dispersive X-ray beam which is finely focussed at the sample and polychromatic, such that the spectral range of the X-rays (50-200eV) is spatially distributed. This allowed us to record X-ray diffraction images from the protein crystals at several wavelengths simultaneously, where the Bragg spot is transformed into either a wavelength streak (DAFS) or string of spots (SMAD).

Since the station ID24 was not equipped for diffraction experiments with protein crystals, a Mar300 image plate system was installed over the first couple of days. Regulating the bent Si(111) polychromator proved to be a delicate operation, requiring a full day per wavelength region to obtain proper alignment and focussing of the X-ray beam. Nevertheless, once in place, the X-ray optics gave stable focal spot sizes of 20-100μm. Calibrating of the spatial resolution of the X-ray wavelengths was done by recording the transmission spectra of a chemical standard (e.g. Fe foil), but as expected, the recording the transmission absorption spectra directly from the crystal samples were fruitless because of their low concentrations of Fe or Hg. For SMAD experiments, a wavelength-selecting finger-slits was positioned in the beam path to select the desired wavelengths and transforms the diffracted wavelength streak into a string of spots.

In order to optimise spot separation between the wavelengths, the image plate was set well back from the sample and this limited the diffraction data to only low or medium resolution. The protein crystals used in this study were selected for their fine mosaic spread, because larger mosaic spreads could cause the

spots from the adjacent wavelengths to overlap. An example of one of the diffraction images recorded is shown in figs 1, 2. The dispersive X-rays in this case were separated into four discrete wavelengths about the Fe K-edge and diffract into a string of four Bragg spots. The protein crystals, however, cracked under the intense X-ray flux of the undulator beam. Despite this difficulty, we were able to record near complete data sets by periodically displacing the crystal and exposing different parts to X-rays. The possibility of cryo-cooling crystals was originally thought to be unsuitable because the process of flash-cooling often increases the crystal mosaic spread. One of the main objectives for follow up experiments is to collect data from cryocooled crystals. Similarily, we have noted that the overall data quality is improved in SMAD images, because the energy resolution is not smeared out by the mosaic spread of the sample as is the situation for the wavelength streak in DAFS data

The SMAD and DAFS images from a cytochrome crystal were integrated using the program DAD which was especially developed for such data (V. Favre-Nicolin). The same SMAD images have also been integrated in parallel using MOSFLM, treating each wavelength separately. The results from the data processing of a cytochrome crystal are listed in Table 1 and they illustrate that high quality data can be obtained using dispersive X-rays. The anomalous signal follows the expected trend, and the results from DAD and MOSFLM are comparable ($R_{merge} \approx 0.150$). The anomalous Patterson maps, however, did not reveal the Fe sites of the cytochrome. This may be because the data is limited to 5.3Å resolution or due to deterioration of the protein crystal in the X-ray beam. Processing of the SMAD data at seven wavelengths for the cellulase derivative crystals at the Hg L_{III}-edge is still underway. Integrating the images has proved to be tricky because of the tightly packed spots which has resulted in imprecisions in refining the detector parameters. We are currently developing a program, SMADSPOT (R. Kahn), which sorts the different spots according to wavelength and should allow more precise orientation matrices to be calculated. The DAD integration protocol is also being improved to cope with tightly packed or slightly overlapping spots.

The experiments completed on ID24 have allowed us to fulfil1 our primary objectives of investigating the feasability of using dispersive X-rays for MAD experiments, as well as, developing the experimental protocols and programs to integrate dispersive X-ray data. We expect to publish the results from these preliminary experiments, and indeed an article describing the DAD integration program will soon be submitted to the Journal of Applied Crystallography. An experimental proposal has been submitted for further experiments to develop the method, in particular the acquisition of high resolution data from cryocooled crystals.

Table 1

Wavelengths		1.7487Å	1.7456Å	1.7424Å	1.7393Å
DAD	R_{sym}	0.069	0.063	0.067	0.071
	R_{anom}	0,089	0,042	0,041	0,039
	% ‹I+›-‹I-› > 3σ	24,3%	4,5%	1,4%	2,2%
MOSFLM	R_{sym}	0,052	0,049	0,046	0,060
	R_{anom}	0,081	0,038	0,033	0,034
	% ‹I+›-‹I-› > 3σ	23,0%	8,7%	2,3%	1,5%

† Abbreviations: MAD (Multiple-wavelength Anomalous Diffraction), DAFS (Dispersive Anomalous Fine Structure), SMAD (Simultaneous Multiple-wavelength Anomalous Diffraction), DAD (Dispersive Anomalous Diffraction).

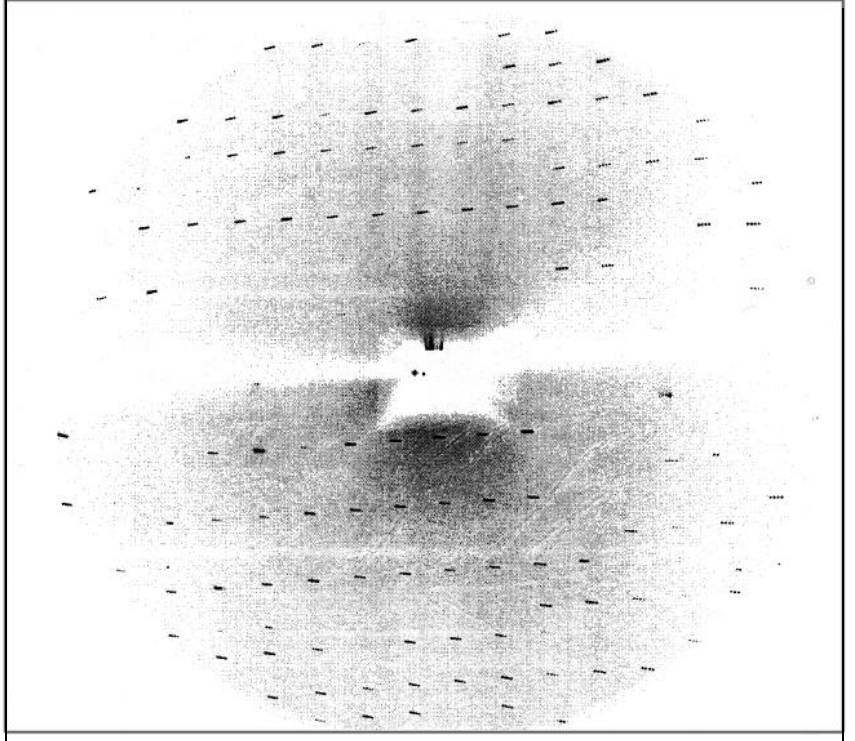

fig. 1 : full oscillation image (10°) for the cytochrome-c7 sample (a=37.6 Å ; b=43.8 Å ; c=45.3 Å)

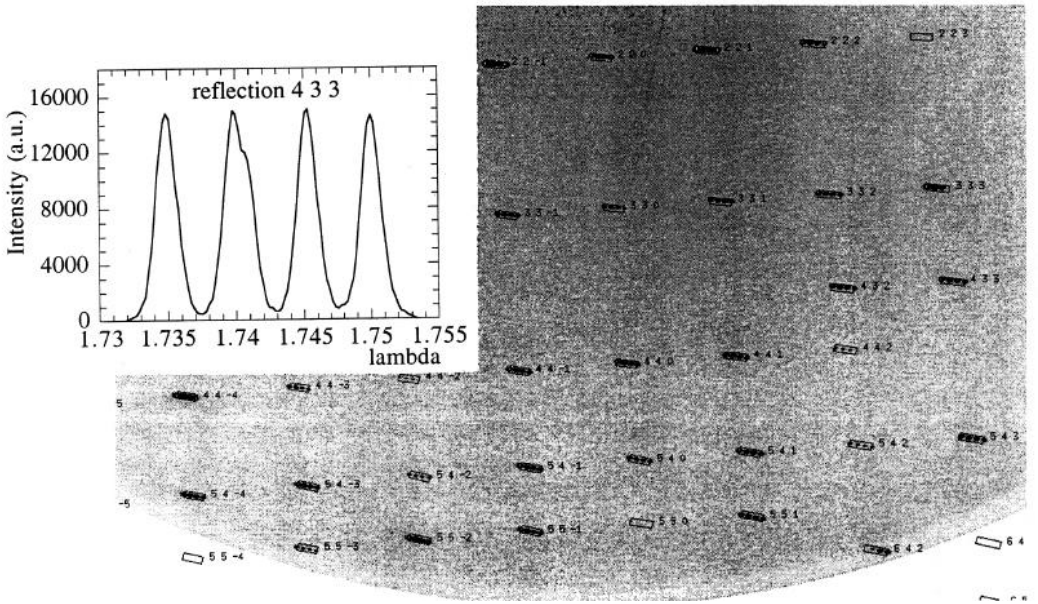

fig. 2 : a portion of the same image, with computed reflection positions. For accurate integration of the almost-overlapping spots, a specific procedure has been developped in the DAD program.