PS01.03.10 A NEW SYNCHROTRON MAD DATA ACQUISITION METHOD: SIMULTANEOUS MULTI-WAVELENGTH ANOMALOUS DIFFRACTION (SMAD). Peter L. Lee* and Craig M. Ogata†, *Materials Science Division, Argonne National Laboratory, Argonne, Illinois 60439, †Howard Hughes Medical Institute, X4, NSLS, Brookhaven National Laboratory, Upton, NY 11973

In recent years, Multi-wavelength Anomalous Diffraction (MAD) phasing has emerged to be a powerful synchrotron technique for solving protein crystal structures. However, a straight forward MAD data collection requires a stable crystal, synchrotron source, and beamline optics. The customized beamlines for fast energy changes, the improvement of flash freezing techniques and the availability of stable long lifetime synchrotron sources around the world have made MAD data measurement a more accessible experiment. Nevertheless, switching among different wavelengths to measure anomalous data sets is still very time consuming and puts great demands on the stability and reproducibility of the monochromator and the synchrotron beam.

We are here introducing a new synchrotron MAD data acquisition method, Simultaneous Multi-wavelength Anomalous Diffraction (SMAD), which can avoid switching energies. SMAD employs a variable bandwidth curved crystal monochromator (polychromator) coupled with an energy selecting grid plate. Our SMAD experiment demonstrates the ability to measure reflections at six different wavelengths and their Bijvoet pairs at the same time. On one diffraction image, we record both the dispersive and Bijvoet information from a myoglobin crystal for MAD phasing.

This work was carried out at both the X4A and the X6A beamlines, National Synchrotron Light Source, Brookhaven National Laboratory, which are supported by the Howard Hughes Medical Institute, and the Division of Materials Sciences and Chemical Sciences of DOE respectively.

PS01.03.11 X-RAY DIFFUSE SCATTERING FROM A LYSOZYME CRYSTAL ANALYSED WITH A RIGID-BODY MODEL OF DISPLACEMENTS. J. Perez, Ph. Faure and J.-P. Benoit, LURE, CNRS-CEA-MENESR, Bat. 209D, Universite Paris-Sud, F-91405 Orsay Cedex, France

X-ray cloudy diffuse scattering from a tetragonal crystal of lysozyme has been collected at room temperature on the wiggler W32 station of LURE synchrotron and interpreted with a simple model of rigid-body displacements. Cloudy diffuse scattering is the part of the scattered intensity which arises from atomic displacements not correlated from cell to cell, and is therefore the signature of intramolecular or molecular correlations.

It is shown here that most of the pattern can be considered as due to independent rigid-body translations and rotations of the protein molecules within the crystal. The normal-mode analysis performed on a single molecule of lysozyme, which accounts only for the intramolecular correlations [Faure et al., 1994], results in a too smooth pattern, underlying the existence of displacements correlated at the molecular scale. By further performing an analysis of the temperature factors of the individual atoms, derived from the crystallographic refinement, it is possible to estimate the meansquare displacement due to the molecular rigid-body motion. The respective values are 0.1Å^{2}for rotations and 0.1Å^2 for translations.

The present diffuse scattering analysis confirms and completes the TLS hypothesis proposed in 1979 by Artymiuk et al., in the sense that it allows to differentiate between a rigid-body rotation movement and a breething movement of the proteins and to estimate the part of the total disorder due to rigid-body tranlations of the whole molecules.

References:
Artymiuk, P.J., Blake, C.C.F., Grace, D.E.P., Oatley, S.J., Phillips, D.C., Sternberg, M.J.E. (1979), Nature, 280, 563-568.
Faure, Ph., Micu, A., Perahia, D., Doucet, J., Smith, J., Benoit, J.P. (1994), Nature:Structural Biology, 1, 124-128.

Detectors & Data Processing I Macromolecular

MS01.04.01 DETECTORS AND DATA PROCESSING: OPTIMISED ANOMALOUS SCATTERING, HIGH RESOLUTION AND DYNAMICAL STUDIES. J R Helliwell, Chemistry Department, University of Manchester, M13 9PL, U.K.

The last 20 years has seen an important evolution of position sensitive detectors for crystallography data acquisition. Film densitometry, MWPC's, TV detectors, image plates and CCDs have been exploited. These devices all have strengths (true counting accuracy/sensitivity, MWPCs; wide wavelength response and high count rate, TVs/IPs/CCDs; large size, IPs) and weaknesses (wavelength range and count rate limits, MWPCs; detector noise, TVs; limited aperture, TVs/CCDs; poor duty cycle, IPs). In recent years very impressive results have been obtained with on-line IP devices, very large IP (Weissenberg) off-line devices, and on-line CCD devices. It has become possible, for e.g., in conjunction with cryoprotection against radiation damage of the protein sample, and/or intense, tunable synchrotron radiation, to readily measure multiple wavelength data sets, reach atomic resolution and record time-slicing dynamical protein crystallographic data. Examples include a brominated oligo-nucleotide MAD study on station 9.5 at Daresbury (IP), a seleno hydroxy methyl bilane synthase (HMBS) MAD study (collaboration with Dr A Haedener) at 9.5 (IP) and ESRF BL19 (CCD), a time-resolved study, also on HMBS, at ESRF BL3 and BL19 (CCD) and data collection on cryocooled concanavalin A to 0.94A (CCD compared with IP) at CHESS. In chemical crystallography examples include use of high photon energy (24 keV) and a CCD at CHESS with a nickel octahexylphthalocyanine and a temperature dependent space group transition. In neutron crystallography the use of IP's has started (e.g. neutron Laue of concanavalin A). Further evolution of detectors is important; the combination of the large aperture of IPs with the better duty cycle of CCDs might be possible with the 'pixel detector', a silicon based device with independent pixel counting chains. The ultimate diffraction measurement scheme of reflections measured only during their active range (seconds of arc rocking widths for lysozyme protein crystals have been realised using microgravity crystal growth) can yield optimal peak to background ratios. New sources beckon. Detector investment needs to be enhanced. Finally data processing at increased reflection measuring rates will be vital for full exploitation of both source and detector developments.

MS01.04.02 COMPARISON OF IMAGING PLATE AND CCD-BASED X-RAY DETECTORS FOR MACROMOLECULES. Y. Amemiya[1] and K. Ito[2], [1]Department of Applied Physics, School of Engineering, The Univ. of Tokyo, Yayoi, Bunkyo, Tokyo 113, [2]Graduate Univ. for Advanced Studies

In x-ray diffraction experiments for macromolecules with use of synchrotron radiation, imaging plate (storage phosphor) detectors[1] and CCD-based x-ray area detectors[2] are currently two of the most widely used x-ray area detectors[3]. Regarding the CCD-based detectors, they are classified into two types; one employs an image intensifier (referred as an "intensifier-coupled CCD")[4,5], and the other employs a tapered optical fiber (referred as a "fiber-coupled CCD")[6,7,8] as a device to de-magnify x-ray image onto a small format CCD. The above three types of the x-ray detectors have high detective quantum efficiency (DQE) (30 - 80 %) and wide dynamic range (4 - 5 orders of magnitude). Besides, they all are well suited to experiments with use of synchrotron radiation, because they don't suffer from count rate limitation,

The intensifier-coupled CCD has a higher DQE compared with the fiber-coupled CCD and the imaging plate. The measured DQEs of the above three types of the detectors will be compared quantitatively and the physics of noise propagation underlying the DQEs of these detectors will be discussed. Other performance characteristics such as dynamic range, linearity of response, and image distortion will be also compared among the three types of the detectors. Finally, advantage and disadvantage of the three types of x-ray detectors ("imaging plate", "intensifier-coupled CCD", and "fiber-coupled CCD") will be discussed from the viewpoint of application for macromolecular crystallography.

1 Amemiya, Y., J. Synchrotron Rad., (1995) **2**, 13-21.
2 Allinson, N.M., J. Synchrotron Rad., (1994) **1**, 54-64.
3 Gruner, S.M., Current Opinion in Struc. Biol., (1994) **4**, 765-769.
4 Moy, J.P., Nucl. Instrum. Methods, (1994) **A348**, 641-644.
5 Amemiya, Y., *et al.*, Rev. Sci. Instrum., (1995) **66**, 2290-2294.
6 Tate, M.W., *et al.*, J. Appl. Cryst., (1995) **28**, 196-205.
7 Thiel, D.J., *et al.*, Rev. Sci. Instrum., (1995) **66**, 1477-1479.
8 Naday, I., *et al.*, Nucl. Instrum. Methods, (1994) **A348**, 635-640.

MS01.04.03 IMAGING PLATE DATA PROCESSING FOCUSED ON CHEMICAL CRYSTALLOGRAPHY. T. Higashi, Rigaku Corporation, 3-9-12 Matsubara-chi, Akishima-shi, Japan 196

Rapid data collection using an area detector developed for protein crystallography is now successfully applied to small molecule crystallography, especially when crystals are unstable. As far as imaging plate data processing is concerned, however, software strategy suited to chemical crystallography is not yet completed.

In a physical sense, diffraction phenomena do not discriminate small molecule or macromolecule crystals, difference is just size of cell dimensions, ie. a protein data acquisition method can be straightforwardly applicable to small molecules, except a few points.

1) Alpha-1 alpha-2 splitting of spots, resulting in re-consideration of a measurement box. One solution could be superposition of two separated spot boxes. Local profile fitting of DENZO may compensate it to some extent, or a dynamic box based on the seed-skewness method would be another possibility.

2) Less dense reciprocal lattice points, causing sometimes difficulty in indexing. Real space indexing would be a solution. On the other hand, an area detector loses benefits of four-circle diffractometry in the points.

1) Less accurate cell constants, arising from rough estimation of diffraction geometry. Direct two-theta measurement of reflections, calibrated with a standard powder sample, may be required.

2) No experimental absorption correction, such as a psi-scan method, available. Since easy DIFABS correction is banned in Acta Crystallographica, an alternative method is highly required. Discussed will be some solutions to those problems.

MS01.04.04 AN EXTREMELY FAST DIRECT PHOTON COUNTING DETECTOR FOR PROTEIN CRYSTALLOGRAPHY. Ng.H. Xuong[1], P. Datte[1], E. Beuville[2], T. Earnest[2],H. Padmore[2], J. Millaud[2], D. Nygrent[2], Department of Physics, UCSD, La Jolla, CA 92093-0359[1]. Lawrence Berkeley Laboratory, Berkeley, CA 94720[2].

A Smart Pixel Array Detector (SPAD) is being designed which will collect a complete set of monochromatic data for protein crystals in 1.5 minutes and a good Laue picture in less than 10 ms. The readout will also allow framing of up to 16 successive Laue pictures with a switching time of less than 100 μs between pictures. The SPAD will consist of 1000 x 1000 pixels of 150 μm x 150 μm in size[1]. The system is being designed around the Column Readout Architecture presently being developed at LBNL[2]. The column readout will allow a photon count rate of 1 million (photons/second) at the pixel and a photon count rate of 8 million (counts/sec/column) total ouput (i.e. up to 8 billion counts per second for the complete detector). Each pixel has its own preamplifier, shaper, discriminator, and a 3 bit prescaler. The material used for the detector will be Si, however we are investigating the use of CdZnTe for measurements that require a larger photon dynamic range or a higher monochromatic photon energy. The data will be stored in real time, in a large histogram memory capable of gathering data for 16 successive pictures. The preliminary results of an 8 x 8 prototype of both Si and CdZnTe will be presented.

[1]N.-H. Xuong, P. Datte, E. Beuville, J. Millaud, D. Nygren, "A Very Advanced Detector for Synchrotron Radiation," Proceedings of the 16th European Crystallographic Meeting (ECM 16), August 1995, Lund, Sweden.
[2]J. Millaud, D. Nygren, "The Column Architecture - A Novel Architecture for Event Driven 2D Pixel Imagers," Proceedings of the Nuclear Science Symposium and Medical Imaging Conference, October 21-28, 1995, San Francisco, CA.

MS01.04.05 THE DEVELOPMENT OF MAD PROTEIN CRYSTALLOGRAPHY AT THE ESRF. A. Thompson, V. Biou ESRF, Grenoble and IBS, Grenoble, L. Claustre, F. Felisaz and A. Thompson EMBL, Grenoble Outstation, A. Gonzalez ESRF Grenoble, current address EMBL Grenoble Outstation, J. Helliwell University of Manchester, J.L. Smith Purdue University and EMBL, Grenoble Outstation, A. Hammersley and P. Thorander ESRF, Grenoble

BL19, has been built on a bending magnet at the ESRF as a collaboration with EMBL Grenoble , and is a dedicated beamline for the measurement of Multi-Wavelength Anomalous Diffraction (MAD) data. The beamline has been operational in commissioning mode since June 1995, and in user mode since September 1995.

The power of the MAD technique for rapidly solving structures using a dedicated beamline with very stable beam is illustrated by the fact that 6 new structures (phased solely using MAD) are already in an advanced state of refinement, with good electron density maps available from other samples. The size of problems successfully tackled has varied from 12 kDa to 39 kDa, with various anomalous scatterers (Se-Met, Fe, Sm, Hg). In favourable cases it has even been possible to solve the anomalous Patterson and examine the initial MAD map during the data collection time. It would be true to say that MAD is now a routine technique at the ESRF for reasonable sized proteins (up to 20 kDa) with several anomalous scatterers (Se-met or good derivatives) and reasonable (a few percent) anomalous signal. With a fast readout detector,powerful local computing permitting online integration and scaling of data, and a combination of direct methods or Patterson search routines, an initial map could be arrived at in 48 hours permitting users to leave the synchrotron with HKL |F| and phi.

Further development at the ESRF includes the use of a CCD based detector for rapid measurement (3.4 s per image to 16 bits, and 0.4 s per image to 12 bits), investigation of the impact of phi-slicing and dynamic range extension on the quality of MAD data (particularly at high resolution), the development of data collection and strategy software to ensure correct coverage of Bijvoet mates, and the development of an undulator beamline for MAD measurements (to be operational by the end of the decade).

The advantages of a high intensity, collimated, stable beam for MAD will be discussed and illustrated with examples of data collected on the beamline using both image plate and CCD detector. Data collection strategy will be discussed for both cryo - protected and unprotected crystals, and future possibilities indicated.