01.X-4 IMAGE PLATE PROTEIN DATA: AN EXAMPLE OF EXPERIMENT WITH A WEISSENBERG CAMERA USING SR IN THE PHOTON FACTORY. By N. Sakabe, National Laboratory for High Energy Physics, Oho, Tsukuba, Ibaraki 305, Japan.

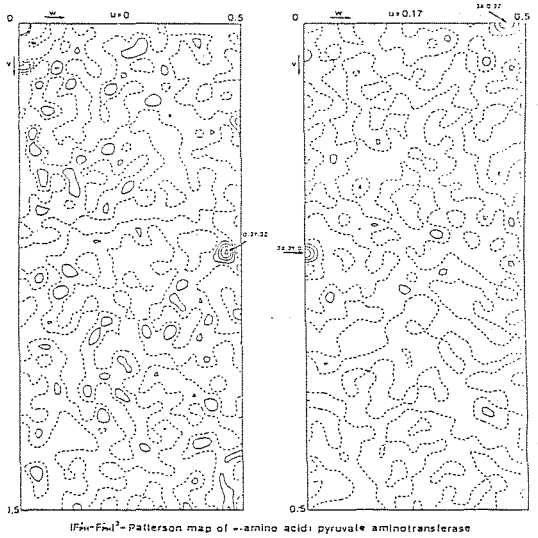
Fuji Imaging Plate(IP) is an area detector based on a phosphostimulable phosphor($BaFBr:Eu^{2+}$) screen to store a two-dimensional image produced by the irradiation of X-rays. The characterization of IP with Fuji computed radiography(FCR) system has been reported by J. Miyahara (J.Miyahara, K.Takahashi, Y.Amemiya, N.Kamiya & Y.Satow, Nucl. Instr. & Meth. Phy. Res. 1986, A246 572-578).

Mersalyl derivative of ω-amino acid:pyruvate aminotransferase crystals were used in this experiment. This enzyme is a tetrameric protein of 172,000 daltons and crystallized in space group I222 with a=124.67,b=137.90 and c=61.45A. The data was collected with a Weissenberg camera (N. Sakabe, J.Appl.Cryst.1983,16 542) with 143.5 mm radius of a cylindlical cassette in combination of IP (HR), when PF was operated at 2.5GeV and 150mA. The intensity data was collected at λ= 1.004A. The oscillation axis was the c-axis (4°/mm), and the oscillation angle was 18°. The exposure was stopped after 12 times oscillation, and exposure time was about 4 min. The total range of 162°were recorded at ten shots with overlapped area 2°. A complete data set was collected with a single

Method/ Scan step	Imaging plate/100μm			Photographic film/25μm		
Miller index	(7 8 1)	(4 5 1)	(5 6 1)	(7 8 1)	(4 5 1)	(5 6 1)
Integrated intensity	135	342	2933	1716	4386	14142
I/I(7 8 2)	1.00	2.54	21.8	1.00	2.56	8.24
Heighest peak value	23	44	440	53	95	206
Background level	4	3	4	29	28	29
S/N ratio	5.75	14.7	110.0	1.83	3.39	7.21
Half width(RxSmm)	0.3x0.25	0.3x0.25	0.3x0.25	0.23x0.2	0.28x0.15	0.30x0.25
Peak size(RxSmm)	1.0x0.6	0.9x0.7	1.3x1.2	0.33x0.3	0.45x0.3	0.53x0.35
Half width area (pixels)	5	6	4	49	50	55
Peak area (pexels)	37	44	100	113	165	256

crystal up to 2.3A resolution and the merge $R(F^2)$ was 0.056. The comparison of diffraction spots on IP and a film is in the Table. In order to know the feasibility, $(F^+ - F^-)^2$ Patterson maps were calculated and heavy atom vectors are clearly appeared even if the occupancy of the Hg is about a half. The absorption correction (C.Katayama, N.Sakabe & K.Sakabe, Acta Cryst. 1972, A28 293-294) is effective since after the correction, R value was reduced from 0.052 to 0.038. We conclude that the combination of the Weissenberg camera and IP is one of the best system for the data collection of protein crystals using SR.

We are grateful to Mr. J. Miyahara of Fuji Photo Film Co., Ltd. for reading out IP with FCR.



$(F^+ - F^-)^2$-Patterson map of ω-amino acid: pyruvate aminotransferase

01.X-5 SYNCHROTRON RADIATION LAUE DATA COLLECTION FOR KINETIC STUDIES. By [2]Donald Bilderback, [1]Keith Moffat, [1]Wilfried Schildkamp, [1]Doletha Szebenyi, [3]Brenda Smith Temple, and [1]Karl Volz.
[1]Section of Biochemistry, Molecular and Cell Biology, [2]Cornell High Energy Synchrotron Source, and [3]Department of Applied and Engineering Physics, Clark Hall, Cornell University, Ithaca, NY 14853, U.S.A.

X-ray diffraction data from single crystals of typical proteins are very weak, numerous, and subject to systematic errors arising from radiation damage. Even with intense synchrotron x-ray sources, exposure times for monochromatic oscillation photographs lie in the range of seconds to minutes. This precludes time-resolved studies on those biochemical systems where lifetimes of structural intermediates are seconds or less, as is almost always the case. If the x-ray monochromator is dispensed with, then a polychromatic x-ray beam falling on a stationary crystal will yield a Laue diffraction pattern (Moffat et al., Science 223, 1423 (1984); NIM 222, 245 (1984); NIM A246, 627 (1986); Helliwell, J. Mol. Struct. 130, 63 (1985)). Such Laue patterns require extremely short exposure times for strongly scattering protein crystals, in the ms to s time range; yield integrated intensities even with a stationary crystal; may be quantitated with very good precision; may contain a substantial fraction of the entire unique data in one pattern; and do not suffer greatly from overlapping orders (Cruickshank et al., Acta Cryst. A, in press; unpublished results of the authors, and of D.W.J. Cruickshank, J.R. Helliwell and colleagues). These properties fit the Laue technique well both for static, and even more so for dynamic, data collection; that is, for time-resolved crystallography on a biochemical time scale.
The principles of Laue diffraction from macromolecular crystals, and preliminary application to time-resolved studies, will be discussed.

01.1-1 GREATLY REDUCED RADIATION DAMAGE IN RIBONUCLEASE CRYSTALS MOUNTED ON GLASS FIBERS.
By John C. Dewan and Robert F. Tilton, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.

We have been engaged in a program of X-ray data collection, primarily from bovine pancreatic ribonuclease-A (RNase-A) crystals, at temperatures ranging from 320K to 98K as part of a study of protein structural dynamics. During the course of investigations at 220K, 180K, 160K, 130K, and 98K we have mounted RNase-A crystals on glass fibers and have noticed that these crystals exhibit almost no radiation damage whereas an RNase-A crystal at 160K that was mounted in a quartz capillary tube showed significant deterioration. Another data set, collected at 103K from a crystal of sperm-whale metmyoglobin mounted on a glass fiber, also exhibited no radiation damage.

RNase-A crystals in 50% MPD:H_2O and maintained at ~273K with an ice bath (MPD = 2-methyl-2,4-pentanediol), can be picked up on a glass fiber that has a ball of uncured epoxy cement near the tip. The glass fiber itself is glued to a brass pin which is mounted on a standard goniometer head. The crystal is pressed into the uncured epoxy and, once adhering to the fiber, the whole assembly is quickly transferred to the diffractometer where the crystal is prevented from drying out by virtue of the nitrogen-gas cold-stream of the low-temperature device. The crystal is not necessarily covered completely by the epoxy.

Data collection from RNase-A at 160K, with the crystal mounted in a quartz capillary tube, showed a 21% decay of the intensity standards after 14.6 days of X-ray exposure. RNase-A crystals mounted on glass fibers, however, showed virtually no decay of the intensity standards even after extensive X-ray exposure. For

example, one crystal of RNase-A was used to collect data from 1.5 to 1.2-Å resolution at 160K. The temperature was then lowered to 98K and data from ∞ to 1.5-Å resolution were collected on the same crystal. After a total of 28.3 days of X-ray exposure the decay in the intensity standards was about 3%. Data from two other RNase-A crystals have also been collected at 180K and 130K from ∞ to 1.5-Å resolution and, again, the decay in the standards after 13.1 days of X-ray exposure was only about 3% in both cases. Data were also collected from an RNase-A crystal at 220K and no radiation damage was observed over an 8.1 day period of X-ray exposure. The structures at each temperature have been refined to current R values of between 14 and 16%.

The reasons for the large reduction in radiation damage that we have observed for RNase-A crystals mounted on glass fibers are not at all obvious but may possibly be linked in some way to the removal of the glass of the capillary tube from proximity to the crystal, to the lack of excess mother liquor surrounding the crystal, or to the exclusion of oxygen from the crystal during data collection. The large reduction in radiation damage appears to be a combination of both the low temperature and the lack of the capillary tube.

01.1-2 USE OF POLYCHROMATIC SYNCHROTRON X-RADIATION IN PROTEIN CRYSTAL LAUE DIFFRACTION.

R Brammer[1], J Campbell[1], I Clifton[1], D W J Cruickshank[1,2] M Elder[1], T J Greenhough[1,3], G Habash[2], M M Harding[4], J R Helliwell[1,2], P Machin[1], P R Moore[1], A W Thompson[1] and T Wan[2].

[1]SERC, Daresbury Laboratory, Daresbury, Warrington WA4 4AD, UK
[2]Department of Physics, University of York, Heslington, York YO1 5DD, UK
[3]Department of Physics, University of Keele, Keele, Staffs, ST5 5BG, UK
[4]Department of Chemistry, University of Liverpool, Liverpool, UK.

The Daresbury SRS wiggler is used to provide an intense, smooth continuum of X-rays between 0.2Å - 2.5Å. Laue patterns can be recorded from protein crystals in approximately 100 milliseconds to 1 second using the SRS wiggler protein crystallography workstation (Helliwell et al (1986) Nucl.Instrum. and Methods A246, 617). On this station (9.6) the vertically diverging beam is collected by a cylindrical focussing mirror set to reflect λ's ≥ 0.5Å. A new workstation is currently under construction on the wiggler line. The design of the new station involves a toroid mirror to focus the vertically and horizontally diverging beam. Laue patterns are expected to be recorded in 1-10 milliseconds. The new station will also be used for point focussed, rapidly tunable monochromatic experiments.

The advantage of using a broad wavelength band is that a large continuous region of reciprocal space is sampled in a single shot. This "traditional" benefit of Laue geometry, taking advantage of the short exposures now possible, is useful for monitoring, in a qualitative way, crystal transitions. This has been used by Hajdu et al ((1986) Biochem Soc Trans) to study an order-disorder-reorder process in crystals of phosphorylase b.

The use of Laue diffraction data in a quantitative way, in difference Fourier maps, has necessitated new data processing software and new theory. The strategy for the processing software (Helliwell (1985) J Mol Struct 130, 63) involves the usual steps of sample refinement, prediction and integration. The single wavelength Laue spots and multi-component Laue spots are treated differently. Several reciprocal lattice points (rlps) stimulated in one spot can be unscrambled using differential film absorption (for the case of double and triple reflection spots). The integrated data are currently being used in three ways. Hajdu et al (pers comm) are using the fractional difference intensity in Fourier maps whereby wavelength dependent parameters cancel out, to study enzyme substrate interactions. Helliwell et al (this abstract) are comparing the data derived from Laue geometry with monochromatic data from the source crystals to check for systematic errors and improve the software; wavelength normalisation procedures have been developed (partly in collaboration with K Moffat). Harding et al (pers comm) is using wavelength normalized Laue data to solve small molecule crystal structures.

New theory has been necessary. Cruickshank, Helliwell and Moffat ((1987) submitted to Acta Cryst) have derived the observed multiplicity distribution of energy overlap spots. It has been established that the proportion of single wavelength spots is never less than 73% and depends on λmax/λmin and the number of Laue spots depends on (λmax-λmin).

01.2-1 FIBER DIFFRACTION AS AN ALTERNATIVE TO PROTEIN CRYSTALLOGRAPHY.

Gerald Stubbs
Dept. of Molecular Biology, Vanderbilt University, Nashville, TN 37235, USA

Many important biological macromolecules, for example actin, myosin, tubulin, flagellin, and the coat proteins of some viruses, form filamentous assemblies with functions specific to those assemblies. Even in cases where these molecules can be crystallized as monomers or small aggregates, it is important to know the molecular structure of the intact assembly in order to understand the function of the molecule. It is therefore necessary to use the methods of fiber diffraction.

The central problem of fiber diffraction is that the random azimuthal orientations of the diffracting particles cause the data to be cylindrically averaged. Considerable information is thus lost; for example, at 3Å resolution the effective number of observable diffraction data for tobacco mosaic virus (TMV) is reduced by a factor of 2.5, and for the bacteriophage Pf1 by 1.7. These factors are much higher for lower symmetry systems such as microtubules.

Multi-dimensional isomorphous replacement, analogous to isomorphous replacement in protein crystallography, can be used to compensate for the loss of information. Large numbers of heavy-atom derivatives are needed, but these numbers can be reduced by taking advantage of the fine splitting present in the diffracted layer lines when, as is usual, the helix in the diffracting system repeats approximately, but not exactly, in a small number of turns. Phases determined by MDIR and layer-line splitting can be refined by density modification (solvent flattening), which provides much more powerful constraints in fiber diffracting systems where the diffracted intensity is continuous along layer