01.X-1 RECENT DEVELOPMENTS IN DATA COLLECTION

TECHNIQUES FOR LARGE UNIT CELLS. By J R Helliwell, Department of Physics, University of York, England and SERC, Daresbury Laboratory, England.

The procedure for the collection of X-ray diffraction data from single crystals of macromolecules has improved considerably. Monochromatic methods have taken advantage of storage ring synchrotron radiation sources. New area detector devices have been used on conventional X-ray sources and the synchrotron to great advantage. These devices include MWPC's (in detector arrays and/or at high pressure), the television system and the imaging plate. Software is being developed to handle these new data. As a result more demanding samples can be studied and/or structures solved more quickly.

Laue diffraction methods using the smooth polychromatic spectrum from synchrotron wiggler sources currently allow sub-second to millisecond sampling of large regions of reciprocal space. Time resolved studies on enzyme crystals have yielded interesting results simply from an examination of order-disorder transitions evident in the Laue patterns. New software strategies and packages for processing Laue data and new theoretical work should allow quantitative structural data to be obtained via, for example, difference Fourier maps.

This paper serves as an introduction and general review of the important topic of macromolecular crystal data collection covered in microsymposium 3.

01.X-2 MULTIWIRE AREA DETECTOR ARRAYS FOR DATA COLLECTION OF CRYSTALS WITH LARGE UNIT CELLS. By N-h. Xuong, C. Nielsen and V. Ashford, Department of Physics, Chemistry and Biology, University of California, San Diego, La Jolla, California, USA 92093.

The Mark II high speed data collection for protein crystallography consisting of two multiwire area detectors and a rotating anode has been used routinely to collect data from crystals with unit cell dimension up to 300 Å.
The strategy for data collection using this system was outlined in another paper (Xuong et al., Acta Cryst., 1985, B4, 267-269). For a crystal with the longest axis d in Å (and no major systematic absence), the crystal to detector distance is: h x a with h = 27.5cm and a = 25cm; therefore, the resolution R_{max} is related to the setting angle (θ_c) of the detector by:

$$R_{max} = \frac{d}{2} \sin \theta_{max} \text{ and } 2\theta_{max} = \theta_c + \arctan \frac{a}{2D}$$

At the highest resolution, a reflection can be measured only if its reciprocal lattice vector makes an angle smaller than $\pm\Delta\chi$ with the equatorial plane where $\Delta\chi$ is defined by:

$$\tan \Delta\chi = \frac{h}{2D \tan \theta_c + a}$$

Data are collected by slowly moving the angle ω while keeping χ and ϕ constant. If $\Delta\chi$ is small, there will be a need for many runs with different χ and ϕ settings in order to get a complete set of data (Xuong et al., J. Appl. Cryst., 1985, 18, 342-350). As an example, let us look at the data from a crystal of Protocatechuate Dioxygenase from P. leparia that has been collected by R. Stalling and M. Ludwig (University of Michigan). This crystal belongs to the $P2_12_12$ space group with a=125Å, b=162Å, and c=85Å. The detectors are set at D=84cm. To collect a 3Å resolution data set requires 4 high resolution runs with the detector angles set at ± 21° (and therefore $\Delta\chi$=±15°) and 2 low resolution runs with θ_c set at ±10°.
Each run covers about 60° in the ω angle with a rotating speed of about 500 sec per degree in the high resolution runs and 300 sec per degree in the low resolution runs. The total data collection time is 44 hours. It takes about 4 additional hours for crystal alignment before the data collection starts. About 180,000 intensity measurements have been made from 34,864 reflections (with only 515 reflections missing). Therefore, in average, each reflection has been measured 5 times. Table I gives a summary of the quality of the data in function of the resolution.

TABLE I

Resolution	Observation	Reflection	Avg. Reps.	Avg. I/σ	R factor in intensity
∞ -5.1 Å	64,000	7385	8.7	15.0	4.6%
5.1-4.0 Å	41,000	7054	5.8	14.0	4.4%
4.0-3.5 Å	32,000	6990	4.6	10.5	5.7%
3.5-3.2 Å	24,000	6822	3.5	7.4	7.3%
3.2-3.0 Å	19,000	6712	2.8	5.0	9.2%

At this time there are at least 6 proteins whose structures have been solved (and published) with data collected on the Mark II system and whose crystals have at least one unit cell dimension above 150 Å. (Glutamine synthetase, Histidine decarboxylase, Aspartate transcarbamoyl transferase, the Elongation Factor and Thymidilate Synthetase).
Work done under the sposorship of the Research Resource Division of the N.I.H. (Grant No.# RR 01644).

01.X-3 PROCESSING OSCILLATION PHOTOGRAPHS OF VIRUS CRYSTAL DIFFRACTION DATA USING THE "AMERICAN METHOD". By M.G. Rossmann, G. Vriend, M. Luo, E. Arnold, G. Kamer, T.J. Smith, Department of Biological Sciences, Purdue University, West Lafayette, IN U.S.A.

Picornavirus crystals are notorious for their sensitivity to x-ray radiation. It is therefore advantageous to avoid setting photographs prior to obtaining the desired data. Indexing correctly the 30,000-40,000 reflections found on the typical 0.3° oscillation film then requires special techniques. Two methods have been developed, which utilize the Cyber 205 supercomputer, to determine the crystal orientation from the reflection pattern on the film. The first program, called "zones", identifies the zone axes corresponding to different sets of concentric ellipses of reflections. The angles between the observed axes is composed with the angles between all possible zone axes with low indices for known unit cell. The crystals orientation matrix can then be calculated from the positions of the identified zone axes on the film.
The second method utilizes manually measured estimates of the crystal orientation to initialize a systematic search of the crystal orientation angles. An attempt is made to refine each combination of angles, and the success of this procedure is used to guide the program to the correct setting matrix.
The resulting crystal orientation matrix from either program is put into the oscillation processing program where the orientation is further refined with alternate cycles aimed at minimizing the distance between observed and predicted positions and optimizing the degree of overlap between observed and predicted reflections. Finally, the reflections are integrated and flagged if any set of error criteria have been exceeded. Data from the processed films is sorted and combined for post-refinement and averaging.