by van der Waals forces only; no short non-bonded distances are observed.

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Use of the Multiwire Area Detector Diffractometer as a National Resource for Protein Crystallography

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Abstract

After many years of development, a high-speed data-collection system for protein crystallography using multiwire area detectors called Mark II has been dedicated as a national resource at the University of California, San Diego. Protein crystallographers can apply for time and come to this resource to collect data. During the last twelve months, this system has been used to collect more than 5 million intensity measurements in 17 different projects. The quality of the data was excellent with data reproducibility around 6% in intensity. The data were of such quality that many groups with good heavy-atom derivatives were able to have the protein structure solved within two months after the data collection.

The Mark II system which can collect data at 100 times the rate of a standard diffractometer consists mainly of a rotating-anode X-ray generator (Elliot GS6) with a graphite monochromator and two multi-wire X-ray area detectors (Cork, Hamlin, Vernon, Xuong & Perez-Mendez, 1975). The crystal is oriented by a three-circle goniostat and can be cooled to 243 K by a dry nitrogen cooling device. The whole system is controlled by a VAX 11/750 computer. This computer not only controls the orientation of the crystal but also analyzes the data from the area detectors to extract right away the integrated intensity of the recorded reflections as the data are being collected. Both the hardware and software of the Mark II have been described in detail elsewhere (Cork *et al.*, 1975; Hamlin, 1982; Xuong, Freer, Hamlin, Nielsen & Vernon, 1978; Howard, Nielsen & Xuong, 1985). We have now published a method or strategy on how to set up the data-collection runs depending on the space group and cell dimensions of the protein crystal (Xuong, Nielsen, Hamlin & Anderson, 1985). The system is kept in running condition by a small crew consisting of one physicist, one electronic technician and one computer programmer. The first-time user is helped by 'in-house' protein crystallographers but can learn to operate the system in a day or two.

Table 1. *Protein crystallography data collected at the UCSD resource during a* 12*-month period* (1983–1984)

Crystal	Group leader(s)	Space group	Cell axes (Å) a	b	c	Number of intensity measurements	Number of data sets*	Number of crystals used	Resolution (Å)	R_{sym} (%)	Structure solution
DNA polymerase	Steitz, Yale Univ.	$P4_3$	104	104	86	320 000	3	4	2·8	6	Solved
Histidine decarboxylase	Hackert Univ. of Texas	$I422$	222	222	107	724 000	3	3	3·0	6	Solved
Aspartate transcarbamylase, active (R) form	Lipscomb, Harvard Univ.	$P3_121$	122	122	156	840 000	3	8	2·8	6	Solved
Manganese superoxide dismutase	Ludwig, Univ. of Michigan	$P4_122$	146	146	56	340 000	3	3	2·5	3	Solved
Histone octamer	Love, Johns Hopkins Univ.	$P3_121$	119	119	103	384 000	3	3	3·0	6	Solved
Trimethylamine dehydrogenase	Matthews, Washington Univ.	P_{21}	147	72 ($\beta = 98°$)	84	1 000 000	3	5	2·5	5	Good electron density map
L-3-Hydroxyacyle coenzyme A dehydrogenase	Banaszak, Washington Univ.	$C222_1$	227	82	124	438 000	4	5	2·8	6	Good electron density map
Iron superoxide dismutase	Ludwig, Univ. of Michigan	$P2_12_12_1$	82	75	71	101 000	1	1	2·0	6	In progress
Tu-elongation factor	Jurnak, Univ. of California, Riverside	$P222$	71	74	171	350 000	3	3	2·8	6	In progress
Erythrocyte nucleosome	Bunick, Oak Ridge National Laboratory	$P2_1$	166	216 ($\beta = 96°$)	66	52 000	1	1	7	10	In progress
Glutamine synthetase	Eisenberg, Univ. of California, Los Angeles	$C2$	236	135 ($\beta = 103°$)	201	113 000	2	4	3·5	6	In progress
Ribulose biophosphate carboxylase	Eisenberg, Univ. of California, Los Angeles	$I422$	149	149	138	150 000	2	3	2·8	7	In progress
Glycogen phosphorylase	Fletterick, Univ. of California, San Francisco	$P4_32_12$	129	129	119	238 000	1	3	2·8	6	Structure refinement
Chicken dihydrofolate	Kraut Univ. of California, San Diego	$C2$	89	48 ($\beta = 125°$)	64	78 000	1	2	1·6	3	Structure refinement
Cytochrome c peroxidase at 258 K	Kraut-Xuong, Univ. of California, San Diego	$P2_12_12_1$	108	77	52	237 000	1	2	2·0	5	Structure refinement
Compound 1 of cytochrome c peroxidase	Kraut-Xuong, Univ. of California, San Diego	$P2_12_12_1$	108	77	52	53 000	1	6	2·4	5	Structure refinement
Phospholipase	Xuong, Univ. of California, San Diego	$P4_12_12$	88	88	105	400 000	2	2	2·8	6	In progress

* The number of data sets includes at least one native data set. The remaining data sets, if any, are for different heavy-atom derivatives.

Once a year (in about July), the resource sends out a request for proposals (to US crystallographers). The proposals, usually in a form of a two- or three-page letter, are examined by an advisory committee consisting of five distinguished protein crystallographers and biochemists. The advisory committee rates these proposals and those with high rating are selected. After an initial adaptive period, we have settled on the selection of one outside group every month. Each outside group will have two weeks to collect data. The remaining time is devoted to preventive maintenance, system improvement and data collection for 'in-house' protein crystallographers. Since each data set (to 3 or 2·5 Å resolution) usually takes about 2 to 4 d, a period of two weeks will allow a group to collect data on a native crystal and many heavy-atom derivatives. It is interesting to notice that each full data set can usually be obtained with only one crystal.

The Mark II system hardware and software have been designed to extract the best intensity measurements out of each crystal. Since reflections from protein crystals usually have a relatively small ratio of intensity over background, we have tried to *reduce* the background by using a graphite monochromator. The background for each reflection is measured at the same detector coordinates as the reflections but in a nearby range of the ω-scanning angles (Xuong *et al.*, 1978; Howard *et al.*, 1985). To reduce statistical fluctuation, the background counts are accumulated 16 times as long as the time used to measure a reflection intensity (Xuong *et al.*, 1978; Howard *et al.*, 1985). Due to the measurement of hundreds of simultaneous reflections at the same time, we can afford to expose each reflection for a long time (from 150 to 300 s for an average intensity measurement as compared with 10 to 20 s on a standard diffractometer with a rotating-anode generator). To correct for absorption and crystal decay, every data-collection 'run' is divided into scaling shifts of 5° rotation in the ω scanning angle and there are a sufficient number

of duplicate measurements from the same reflection or symmetry-related ones to permit the precise determination of the scale constants by least squares (Xuong *et al.*, 1978; Howard *et al.*, 1985). Since each scale constant applies to a group of reflections collected on each detector that is localized both in reciprocal space and in measurement time, this method is very similar to the local scaling method mentioned by Matthews & Czerwinski (1975), but it also compensates for crystal decay due to radiation damage. However, the main reason for the quality of the intensity data is that each reflection (or its symmetry-related ones) is measured on average from 5 to 10 times. Therefore, if one defines the data reproducibility to be

$$R_{\rm sym} = \sum_{h,k,l} \sum_{i=1}^{N} |\langle I(h,k,l)\rangle - I(h,k,l)_i| / \sum_{h,k,l} \sum_{i=1}^{N} I(h,k,l)_i,$$

where the inner summation is over all measurements of each reflection or its symmetry-related one, then the precision on the average intensity which will be used for phase refinement or electron-density-map calculation will be $R_{\rm sym}/\sqrt{N}$. For example, if $R_{\rm sym} = 6\%$ and $N = 9$ then the average intensity precision will be 2%.

As shown in Table 1, the Mark II system was used during the last twelve months to collect more than 5 million intensity measurements in 17 different projects. The quality of the data was excellent with data reproducibility around 6% (in intensity) for most crystals. The data were of such quality that many groups, with good heavy-atom derivatives, were able to have the protein structure solved within two months after the data collection. Often the electron density map was computed using phase information derived from only one heavy-atom derivative.

In conclusion, our experience has shown that a high-speed data-collection system using a rotating-anode X-ray generator and two multiwire area detectors can be used quite effectively as a national resource. We are planning to add three more chambers to the system making it very efficient to collect data even for crystals with cell dimensions as large as 300 Å.

A few practical details we would like to mention are that the monochromator effectively reduces X-ray damage on the crystal and that the cooling device can sometimes dramatically prolong the life of a crystal (using 278 K instead of room temperature, for example). During the last twelve months, the down-time of the system was kept to less than 20%, most of which was due to troubles with the rotating-anode X-ray generator.

Because the data are available immediately in the final form of integrated intensity measurements, a resource using a rotating-anode X-ray generator and multiwire area detectors is, we feel, preferable to the one with a storage ring and a rotation camera set-up (Greenbough & Helliwell, 1983). It takes time to scan and analyze films from a camera; also the film method is much less precise than a method using a photon-counting detector. Of course one could and should use area detectors at a storage ring too (Greenbough & Helliwell, 1983). We feel, however, that a resource with a rotating-anode and multiwire area detectors is optimum for data collection for most protein structure solutions because it can usually extract more than enough data from a single crystal and because it is much less expensive to operate. A resource with area detectors at a storage ring should be reserved for special projects like time-resolved crystallography (Bartunik, 1983; see also Bildenback, Moffat & Szebenyi, 1984) or phase determination using data from X-rays of different wavelengths (Phillips & Hodgson, 1980; Arndt, Greenbough, Helliwell, Howard, Rule & Thompson, 1982).

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