Journal of
Synchrotron Radiation
ISSN 0909-0495
Editors: **Å. Kvick, D. M. Mills and T. Ohta**

Crystallographic data collection using a 0.22% bandwidth multilayer

Ulrich Englich, Alexander Kazimirov, Qun Shen, Don H. Bilderback, Sol M. Gruner and Quan Hao

Journal of
Synchrotron Radiation

ISSN 0909-0495

Received 9 November 2004
Accepted 24 January 2005

Crystallographic data collection using a 0.22% bandwidth multilayer

Ulrich Englich, Alexander Kazimirov, Qun Shen, Don H. Bilderback, Sol M. Gruner and Quan Hao*

Cornell High Energy Synchrotron Source (CHESS), Cornell University, Ithaca, NY 14853-8001, USA. E-mail: qh22@cornell.edu

To bridge the gap between traditional multilayer and crystal optics a high-resolution multilayer monochromator with a bandwidth of 0.22% has been designed and installed on a bending-magnet beamline (F3) at the Cornell High Energy Synchrotron Source (CHESS) to provide an unfocused monochromatic X-ray beam for protein crystallography experiments. Crystallographic data of excellent quality from a medium-sized protein, Concanavalin A, were collected and processed using standard crystallographic programs. The data were successfully used for a structure solution and refinement. The flux from the multilayer monochromator is enhanced, relative to that from a flat Si(111) monochromator, by a factor of 5; consequently, data collection is faster and/or smaller samples may be used. At the same time, the bandwidth is narrow enough to avoid streaked spots. This experiment suggests that multilayer optics may play a valuable role in satisfying the demands of the structural biology community for rapid X-ray data collection, particularly at under-utilized bending-magnet beamlines.

Keywords: X-ray optics; multilayer optics; monochromators; protein crystallography; macromolecular crystallography.

1. Introduction

The increasing pressure for beam time at synchrotron facilities, especially in the field of macromolecular crystallography, has resulted in demands for ever faster and more efficient technology for performing the experiments. Advancements in the development of fast highly sensitive CCD area detectors, beamline automation and graphical user interfaces have improved the efficiency of the diffraction experiment. Although sensitivity of the detectors has improved data quality and reduced the time it takes to acquire an image (Tate *et al.*, 1995), the speed of the experiment is ultimately limited by the X-ray flux at the crystal. Typically, the X-rays are passed through a silicon monochromator consisting of a pair of vertically diffracting Si(111) crystals with a bandwidth of less than 0.02% of the energy of the beam, which is a smaller bandwidth than is required for a monochromatic oscillation experiment with typical macromolecular crystals. The efforts at CHESS and other synchrotron sources (Deacon *et al.*, 1998; Doing *et al.*, 1998; Berman *et al.*, 1997) have focused on the optimization of the bandwidth of the X-ray beam: wider bandwidth optics provide a higher flux which allows for faster data collection and/or the use of smaller samples. An earlier experiment was performed at CHESS using a 2% bandwidth multilayer (ML) at the D1 station, an unfocused bending-magnet beamline (Deacon *et al.*, 1998). Diffraction data were collected at room temperature from tetragonal hen egg-white lysozyme (unit-cell dimensions 79.0 Å, 79.0 Å, 38.0 Å) and crystals of human methylthioadenosine phosphorylase (122.5.0 Å, 122.5 Å, 45.2 Å) at 100 K. The images consisted of radially streaked diffraction spots, owing to a convolution of the crystal mosaicity, the beam divergence and the wavelength bandwidth. So far, there has been no ideal way of modeling the radially streaked spot profiles.

In the present study, narrower bandwidth multilayers were used to avoid streaked spots. The multilayers were the result of a collaboration between Osmic Inc. and CHESS. The goals of this collaboration were the development of a variety of new synchrotron radiation ML optics with a variety of bandwidths, the measurement of their main X-ray characteristics at CHESS beamlines to allow further optimization of ML technology and, finally, field tests in real synchrotron experiments. Macromolecular crystallography of small- and medium-unit-cell crystals was the main motivation behind narrow-bandwidth ML optics development.

Here we present the use of a high-resolution ML optics with a narrow bandpass of 0.22% to collect data from a medium-sized protein, Concanavalin A (unit-cell volume 480000 $Å^3$).

Data were processed using standard crystallography programs and compared with data of the same protein taken using a Si(111) monochromator.

2. Experimental

2.1. Sample preparation

Concanavalin A (Amersham Pharmacia 17-0450-01) was crystallized as described previously (Greer *et al.*, 1970) utilizing the dialysis method in a TRIS buffer at pH 6.5 in the presence of Mn^{2+} and Ca^{2+} ions. After several days, crystals of size 0.2–0.4 mm could be obtained which were used in the following experiments. Under these conditions, saccharid-free Concanavalin A crystallizes in the orthorhombic space group *I*222.

2.2. Multilayer experiment and data processing

The diffraction data were taken at the F3 bending-magnet station at CHESS. The storage ring was operated at 5.3 GeV with a positron current of 165–120 mA over the life of a fill.

For this experiment, a pair of Al_2O_3/B_4C multilayers produced by Osmic Inc. with an energy resolution of 0.22% were used (Martynov *et al.*, 2003). The high energy resolution was achieved by the combination of a low-contrast (close density values) material pair and a large number of reflecting layers, an approach first suggested by Morawe *et al.* (2001). The multilayers consist of 800 bi-layers with a *d*-spacing of 27 Å that have been deposited on 30 mm-thick substrates to avoid the bending of the substrate owing to the strain built into the multilayer during the deposition. The size of the substrate was 100 mm × 40 mm. The 'intrinsic' rocking curve was measured by using a multicrystal set-up based on a Si(111) upstream monochromator and a pair of Si(004) channel-cut crystals in a dispersive arrangement. The result presented in Fig. 1 shows a maximum reflectivity of 51% and a FWHM of 10.5 arcsec. Note that the latter value is comparable with the width of the rocking curve from a perfect Si(111) crystal.

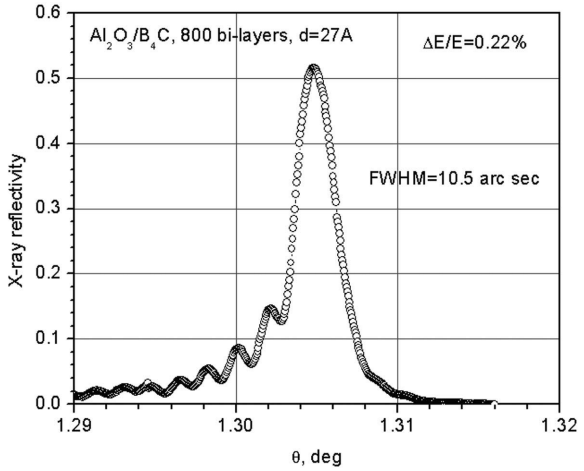

Figure 1
X-ray reflectivity from the Al_2O_3/B_4C multilayer with an energy resolution of 0.22%. The multilayer consists of 800 bi-layers with *d*-spacings of 27 Å that have been deposited on 30 mm-thick substrates. The size of the substrate is 100 mm × 40 mm.

Table 1
Data-processing statistics.

Values for the data in the highest resolution shell are shown in brackets.

	ML022	SI111
Space group	*I*222	*I*222
Unit-cell parameters (Å)		
a	61.73	61.64
b	85.98	85.81
c	89.33	89.04
λ (Å)	1.236	1.236
Temperature (K)	100	100
Detector distance (mm)	133.6	130.9
Oscillation range (°)	1.0	1.0
Exposure (s)	30	180
Number of images	144	144
Resolution (Å)	30–1.90 (1.93–1.90)	30–1.90 (1.93–1.90)
Unique reflections	17992	18897
Multiplicity	4.0 (2.8)	4.9 (3.5)
$I/\sigma(I)$	40.3 (30.6)	42.9 (19.4)
R_{merge} (%)†	5.2 (6.6)	5.0 (11.5)
Completeness (%)	93.8 (95.3)	99.5 (96.5)

† $R_{merge} = \Sigma_{hkl}\Sigma_j|I(hkl)_j - \langle I(hkl)\rangle|/\Sigma_{hkl}\Sigma_j I(hkl)_j$. $I(hkl)_j$ is the observed intensity of the *j*th reflection, and $\langle I(hkl)\rangle$ is the mean intensity of reflection *hkl*.

For the crystallographic experiment, an energy of E = 10.033 keV, λ = 1.236 Å, was chosen owing to mechanical limitations in the monochromator box. X-ray data were collected at 100 K from a crystal of ∼0.4 mm × 0.2 mm × 0.15 mm in size. An unfocused beam was used collimated to 0.3 mm in diameter to collect data with a Quantum 4CCD detector (ADSC); the exposure time was 30 s at 1° oscillation per image.

For comparative purposes a data set was collected from a crystal of similar size at 100 K with a Si(111) monochromator in place at the same energy. For satisfactory images the exposure time had to be extended to 180 s per frame, *i.e.* six times that of the ML experiment, to compensate for the reduced flux of the Si monochromator.

The data were processed using *DENZO* and scaled using *SCALEPACK* (Otwinowski & Minor, 1997). Table 1 summarizes data-collection and processing parameters.

2.3. Structure solution and refinement with the ML data

Concanavalin A is a saccharide-binding metalloprotein of molecular weight 25 kDa with 237 amino acids in the asymmetric unit. The structure was solved in a straightforward manner using *MolRep* (Vagin & Teplyakov, 1997) as part of the CCP4 program package (Collaborative Computational Project, Number 4, 1994). The initial model was obtained from an earlier publication of a high-resolution data set (Deacon *et al.*, 1997), PDB entry 1NLS. Water molecules were removed and all atoms were set isotropic. A single solution was found with satisfactory indicators (*R*-factor of 0.393 and correlation coefficient of 0.568). Subsequent refinement of the model resulted in further improvement and an *R*-factor of 0.24 and R_{free} of 0.293. Solvent water molecules were added using the program *ARP/wARP* (Lamzin & Wilson, 1997) with final *R*

values of R = 0.159 and R_{free} = 0.219 for 1809 protein atoms, two metal atoms and 205 waters.

3. Discussion

The flux for X-rays from the multilayer monochromator is enhanced, relative to that from a flat Si(111) monochromator, by a factor approximately equal to the ratio of the bandwidths and reflectivities, *i.e.* 0.0022 × 0.51 × 0.51/(0.00014 × 0.9 × 0.9) = 5, the reflecting power bandwidths (0.0022 for the ML and 0.00014 for the silicon optic) multiplied by an average reflectivity (0.9 for silicon, 0.51 for the ML) squared for the two bounces taken through each monochromator element. The silicon values are consistent with dynamical theory calculations (Batterman & Bilderback, 1991) of peak reflectivity over the Darwin width of reflection and the ML values were measured experimentally. The intensity gain of 5 agrees well with the measured flux ratio; consequently, data collection is faster and/or smaller samples may be used.

Fig. 2 shows a typical diffraction image taken from Concanavalin A using the narrow-bandwidth ML. It shows a strong diffraction pattern with high intensities up to the edge of the detector (1.9 Å). Images of frozen crystals were collected in a conventional manner using the oscillation method, and profiles were comparable with those collected using Si(111) monochromatic X-rays. In contrast to the earlier experiments with 2% bandwidth ML (Deacon *et al.*, 1998) the profiles of the reflections obtained using the narrower 0.22% bandwidth ML are not noticeably elongated, even though data were collected at 100 K (freezing usually increases mosaic spread of the crystal). The images can be integrated with well established 'single wavelength' processing software such as *DENZO* **(**Otwinowski & Minor, 1997**)** giving excellent merging R values for the observed orthorhombic symmetry. Radiation damage was modest as indicated by a small increase of R_{merge} from 0.044 (the first image) to 0.055 (the last image), and also within the range observed for this particular crystal by using Si(111) radiation. The mosaicity at the beginning was refined to 0.8° and increased moderately to 1.2°.

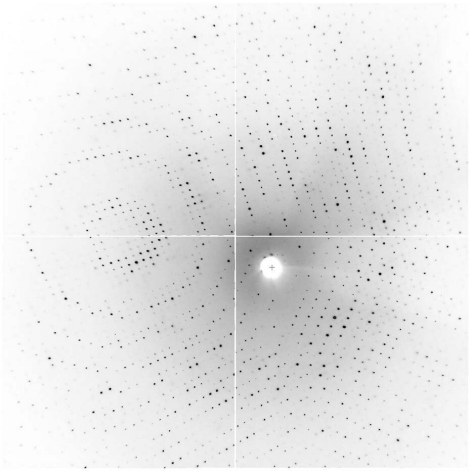

Figure 2
Oscillation image collected using a 0.22% bandwidth multilayer monochromator from a crystal of Concanavalin A at 100 K (1° oscillation in 30 s).

One of the main motivations behind our work was to expand the possibilities to perform crystallography at underutilized bending-magnet beamlines. However, these ML optics can work with more powerful sources considering the small size of the beam and using a mirror to reduce thermal load. For example, we are using similar optics at a wiggler source under significant thermal load. The analysis of the behavior of the multilayer optics under high heat load will be published separately (Kazimirov, unpublished results). We expect that these particular ML optics can be used up to 20 keV without deteriorating their reflectivity. Further experimental testing is required to verify this.

In the kinematical limit, the energy resolution of a multilayer with N periods is proportional to $1/N$ (Nagel *et al.*, 1982). The excellent resolution of these multilayers was achieved by increasing the number of layers to 800 (*versus* typically 200). It was mentioned by Martynov *et al.* (2003) that the limit for these layer materials is 1500 bi-layers, but the deposition of more than 1000 periods is currently not practical owing to the problems of keeping the d-spacing constant during longer deposition time. The current wavelength resolution of 0.22% is perfectly adequate for monochromatic data collections but not suitable for multiwavelength anomalous diffraction experiments.

4. Conclusions

The results here show clearly that the new ML optics that we used in this experiment provide a five-fold gain in flux while still allowing well established procedures and software to be used to collect and process protein crystallography data. In comparison with the ML optics used in the earlier experiments with bandwidths of 1–2%, the new ML optics would allow the collection of diffraction data for specimens with larger unit cells of up to ∼800 Å. This will be subject to investigations in forthcoming experiments. The new optics significantly enhances opportunities for bending-magnet stations to compete with insertion-device beamlines, and to satisfy the increasing demands of protein crystallography.

High-resolution multilayers used in this experiment were produced by Osmic Inc. as a result of collaboration between Osmic and CHESS. We thank Marian Szebenyi, Chris Heaton, Qingqiu Huang, Xianghui Xiao and Tom Krawczyk for their help in setting up the F3 station for macromolecular experiments. This work is based upon research conducted at the Cornell High Energy Synchrotron Source (CHESS), which is supported by the National Science Foundation and the National Institute of General Medical Sciences through NSF grant DMR-0225180, using the Macromolecular Diffraction at CHESS (MacCHESS) facility, which is supported by award RR-01646 from the National Institutes of Health, through its National Center for Research Resources.

References

Batterman, B. W. & Bilderback, D. H. (1991). *Handbook on Synchrotron Radiation*, Vol. 3, edited by G. Brown and D. E. Moncton, pp. 105–153. Amsterdam: Elsevier Science.

Berman, L. E., Yin, Z., Dierker, S. B., Dufresne, E., Mochrie, S. G. J., Tsui, O. K. C., Burley, S. K., Shu, F., Xie, X., Capel, M. S. & Sweet, R. M. (1997). *AIP Conf. Proc.* **417**, 71–79.

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Deacon, A. M., Appleby, T., Bilderback, D. H., Ealick, S. E., Fontes, E. & Thiel, D. J. (1998). *J. Synchrotron Rad.* **5**, 494–496.

Deacon, A. M., Gleichmann, T., Kalb, A. J., Price, H., Raftery, J., Bradbrook, G., Yariv, J. & Helliwell, J. R. (1997). *J. Chem. Soc. Faraday Trans.* **93**, 4305.

Doing, P., Kycia, S. & Shen, Q. (1998). *Proc. SPIE*, **3448**, 32.

Greer, J., Kaufman, H. W. & Kalb, A. J. (1970). *J. Mol. Biol.* **48**, 365–366.

Lamzin, V. S. & Wilson, K. S. (1997). *Methods Enzymol.* **277**, 269–305.

Martynov, V. V., Platonov, Yu., Kazimirov, A. & Bilderback, D. H. (2003). *Proc. SPIE*, **5195**, 46–53.

Morawe, C., Peffen, J.-C., Ziegler, E. & Freund, A. K. (2001). *Proc. SPIE*, **4145**, 61–71.

Nagel, D. J., Gilfrich, J. V. & Barbee, T. W. Jr (1982). *Proc. SPIE*, **315**, 110–117.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Tate, M. W., Eikenberry, E. F., Barna, S. L., Wall, M. E., Lowrence, J. L. & Gruner, S. M. (1995). *J. Appl. Cryst.* **28**, 196–205.

Vagin, A. & Teplyakov, A. (1997). *J. Appl. Cryst.* **30**, 1022–1025.