MULTILAYER MONOCHROMATORS AND SUPERMIRRORS FOR NEUTRON PROTEIN CRYSTALLOGRAPHY USING A QUASI LAUE TECHNIQUE

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BNL--47954
DE93 001654

ABSTRACT

The major problem in neutron protein crystallography is the low flux of present neutron sources. The collection of a data set using a conventional rotation technique and a 2D detector takes many weeks. One way to increase the flux at the sample is to increase the wavelength bandwidth. The conventional technique using a standard monochromator like Cu or Be has a typical bandwidth of 1%. A multilayer monochromator (1) can however be tailored to give bandwidth up to 25% or even larger and have an angular acceptance angle of $\sim .3\%$. This can be achieved by a multiple spacing multilayer. In this case the diffraction geometry is such that the reflection in diffraction condition is 'scanned' by the $\Delta\lambda$ and not by rotating the reciprocal lattice point through the Ewald sphere (2). Wavelength bandwidth of 10% satisfy this condition. It is important to tailor the bandwidth to the size needed by the extend of the reciprocal lattice 'point' to minimize background. The intensity of the reflection is proportional to the $\Delta\lambda r$ that just covers the width of the reciprocal 'point' while the background is proportional to the full wavelength spread $\Delta\lambda$ that hits the crystal. The conventional Laue technique is not suitable since it increases the background by the ratio of $\Delta\lambda/\Delta\lambda r$, a ratio that can easily reach multiples of 10.

2.0 Quasi Laue Neutron Diffraction

It has long been recognized that neutrons provide an excellent probe to use in analyzing the atomic and molecular structure of proteins.³ To improve the utilization of neutrons, position-sensitive detectors⁴ and efficient monochromators¹ have been developed to counter the effect of the relatively low thermal neutron flux available even at the most optimum high-flux beam reactors. The development of the position-sensitive ³He counters with their limited resolution of ~ 1.3 mm and the development of multilayer monochromators with their inherent large but adjustable $\Delta\lambda$ have prompted this re-evaluation of data collection techniques. For proteins which are often weak scatterers, the accurate integration of reflections is particularly important because of the large background produced by the significant incoherent scattering of hydrogen atoms.

2.1 Neutron Protein Crystallography.

The collection of a conventional protein data set takes many weeks. In our case⁸ we use a position sensitive detector⁴ of 20 * 20 cm with a resolution of 1.1mm and a sample to detector

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distance of ca 600 mm. We use often a Be monochromator set to a wavelength of 1.3Å and a collimator with a beam divergence of .30°. The crystal is then rotated in a normal beam technique with a typical step size of 6 minutes. To collect a full data set we use about 20 different detector settings depending on the crystallographic resolution required and the space group of the particular sample. An increase in flux and the use of multiple or larger detectors obviously would decrease the data collection time manifold.

2.2 Quasi Laue Diffraction Geometry

One way to increase flux is to use a larger wavelength band width by using multilayer monochromators^{6,7,8} and choosing a quasi Laue diffraction technique. An inspection of the Ewald sphere construction² as depicted in Fig. 1 shows the reflection condition for a large wavelength band, in this case diffraction conditions are met while the reciprocal lattice points reside in the shaded region. The diffraction condition for a particular reflection is completely satisfied as long as the shaded region is as wide as the extend of the reciprocal point. While we refer to a reciprocal lattice point - this point is actually extended due to crystal mosaic and crystal size and in angular coordinates is of the size of a few tenth of a degree. The peak intensity is therefore proportional to this reflection width while the background is proportional to the whole width of the $\Delta\lambda$ band used. It is therefore advantageous to match the $\Delta\lambda$ to the characteristics of the sample to achieve the best peak to background ratio. This is important, since proteins have an inherently large background due to the high incoherent scattering from the many hydrogen atoms present (about half the atoms in proteins are hydrogens). This background consideration is the reason while a Laue technique is not suitable for neutron protein crystallography.

Figure 1

Figure 1. Ewald-sphere construction showing the width of a reflection due to $\Delta\lambda$, the wavelength bandwidth. The limiting spheres for the smallest ($\lambda_{\min}$) and the largest wavelength ($\lambda_{\max}$) are drawn. Diffraction occurs at any point in the shaded regions. The large shaded region at the top is due to diffraction in the 'antiparallel' mode. The shaded region on the bottom represents diffraction in the parallel or focusing mode. The vector length v_M is given by the lattice spacing of the monochromator selecting the radiation. On rotation, the reciprocal-lattice vector v (of the sample) will be in reflecting position from P^1 to P^3 over the rotational width $\Delta\psi$ and will produce diffraction over an angle $\Delta 2\theta$. $\Delta\psi$ and $\Delta 2\theta$ vary as a function of reciprocal-lattice length v and layer-line height τ (τ is perpendicular to the plane of the drawing). The diffraction process is mapped on the right side as a representative function of ψ and 2θ . α is the angle formed between the diffraction line and the horizontal axis (2θ). The reflection length l is the length of the distribution at half-height measured in degrees.

To select the $\Delta\lambda$ desired the differentiated Bragg equation has to be considered to choose the appropriate conditions and $\Delta\lambda = \lambda\Delta\theta \cot \theta_m$, where $\Delta\theta = \epsilon$ is the beam divergence and θ_m is the Bragg angle of the monochromator. With the monochromator set for a wavelength λ with a Bragg angle θ_m , the extreme path of the neutrons in the horizontal plane are then at angles $\theta_m + \Delta\theta$ and $\theta_m - \Delta\theta$. These extreme rays correspond, therefore, to wavelength $\lambda_{\min} = \lambda(1 - \Delta\theta \cot \theta_m)$ and $\lambda_{\max} = \lambda(1 + \Delta\theta \cot \theta_m)$. The monochromator, therefore, sorts out the wavelength according to angle with the longer wavelength emerging at higher angles. The effect of $\Delta\lambda$ on the diffraction condition is again depicted by an Ewald-sphere construction (Fig. 1). In this case, the two limiting spheres have radii $r_1 = 1/(\lambda - \Delta\lambda)$ and $r_3 = 1/(\lambda + \Delta\lambda)$. The equatorial circles will intersect at two points, the origin of the reciprocal lattice at 0 and the point M , the so-called focusing position at the end of the reciprocal-lattice vector v_m of the monochromator spacing. In this construction, the required diffraction conditions are satisfied for all points in the shaded area. A reciprocal-lattice vector v rotating perpendicular to the rotation axis ψ located at 0 will produce diffraction from point P_1 to P_3 ; during the rotation $\Delta\psi$, the resulting rays will diverge at an angle $\Delta 2\theta$.

The polychromaticity of the beam results, therefore, in the spreading of the intensity of an ideal reflection along a line of length l inclined at an angle α . The magnitude of the length l and the angle depend on the reciprocal-vector length (e.g. hkl) and the basic diffraction condition. This diffraction behavior is again best depicted in the ψ - 2θ space with a reflection of length l inclined at an angle α .

Tests of such an arrangement using 60 Å multilayers on the membrane diffraction station H3B at the HFBR at BNL showed that a 3 fold gain in diffraction intensities is readily achievable by comparison of some low order reflections (6,0,-3 etc) from a large myoglobin crystal to the same data measured with a Zn monochromator. This gain of a factor of 3 can be improved with multilayers permitting a larger acceptance angle matching the protein crystals mosaic. To achieve this a multilayer of high reflectivities with different d spacings is required. For an acceptance angle of 0.3° a Ni -Ti multilayer with 9 variable d spacing from 50 to 70 Å is needed. To give equal reflectivities the number of layers needed for each d spacing is decreasing as the layers become thicker. To achieve this, experience has shown that a total of close to 1000 layers are needed, decreasing from 136 layers for the 50 Å spacing to 70 layers for thicknesses of 70 Å. (A.Saxena private communication). A number of scientists like

Ebisawa, Mezei, Majkrzak and Scharpf have proposed similar algorithms that produce large $\Delta\lambda$ with given beam divergences. Extrapolating from the present measurements with multiple d spaced multilayers it can be expected that a seven fold increase in intensities can be achieved.

2.3 Focusing Multilayers

I have also tested a number of focusing arrangements using a bender similar to the device originally tested in my laboratory by Nunes and Zaccai and used by Saxena and Majkrzak. Using a multilayer with a d spacing of 60 Å in a monochromatic beam of 1.6 Å with a beam divergence of $.3^\circ$ I obtained a horizontal focusing effect from an original beam size of 6 mm to 3 mm measured at half height with an increase in peak intensity from 10000 counts to 16200. Adding vertical and horizontal focusing in a two mirror arrangement or using a toroid figure will increase the effective flux by at least a factor of four. The beam divergence acceptable by the protein crystal determines largely the amount of effective focusing that can be achieved and in this case with a protein crystal of myoglobin or plastocyanin beam divergences of $.3^\circ$ were dictated by the crystals mosaic. The multilayer was focused onto the detector with a focusing distance of 110 cm with the crystal at 50 cm from the multilayer.

The incident beam contained some harmonic components with a $1/2 \lambda$ contribution of 12% - the multilayer removed this component by a factor better than 3 orders of magnitude. These observations demonstrate that a conventional protein spectrometer using a monochromator like Zn etc can be improved by adding a focusing multilayer. Such a multilayer should be of the multi d spacing type to accept the beam divergence that yields optimum performance.

Figure 2 and Figure 3

3.0 Peak Shapes

The effects of diffraction conditions on the observed peak shape as a function of crystal rotation has been described by a number of authors (2, 9-12). In the case of the above proposed quasi Laue technique a careful analysis of the effect of $\Delta\lambda$ on the peak shape is particularly important since it considerably smears the diffraction peaks and can easily lead to reflection overlap. It is therefore worthwhile to recapitulate the effect of $\Delta\lambda^2$. Fig. 2 depicts a typical spot shape as a function of rotation angle and 2θ , including effects like crystal mosaic, beam divergence, crystal size and detector resolution. This reflection shape can be characterized by the extend of the elliptical axes and the inclination angle α of the ellipse. In addition the effect of $\Delta\lambda$ has to be evaluated as a function of 2θ (Fig. 3).

This $\Delta\lambda$ effect on the diffraction peak shape is convoluted with the peak shape derived for crystal mosaic and detector resolution. The resultant peak is much larger and changes its shape and size as a function of 2θ . A knowledge of these diffraction peak shapes is necessary to determine optimum diffraction conditions without causing overlapping reflections. In addition these peak shapes can directly be used to act as masks in integration algorithms. In practice, the initially theoretical precalculated shapes are updated with data from the observed reflection profiles to assemble masks that closely represent the actual data. These masks are then used to delineate and integrate weaker reflections to yield intensities with an optimum peak to background ratio.

4.0 Protein Crystallography at Spallation Sources

The Quasi Laue technique is ideal for use at pulsed neutron sources since it allows the use of a low energy resolution configuration using a long proton pulse width (poisoned hydrogen moderator) with the resultant high flux. Such a proposed instrument is depicted in Fig 4 with a total beam length of 7 meters. For a wavelength band width of 4Å (1Å to 5Å) and a $\Delta\lambda$ of .4Å, data is collected in a fixed time interval proportional to $\Delta\lambda$ or as a function of $\Delta\lambda/\lambda$. The initial radiation burst with its large gamma component is suppressed by the phased To chopper and the long wavelength cut is achieved by another phased chopper T_1 . The exact λ window (1 to 5Å) is gated electronically at the detector.

The focusing optical system uses a toroid supermirror^{13,14,15} and a bendable cylinder (supermirror). This optical system is designed to focus neutrons with a divergence angle of .3° to match the typical acceptance angle of large protein crystals. To collect as many reflections as possible a large cylindrical gas wire detector is planned with a height of ~ 17 cm¹⁶). Such a detector is divided into 4 active segments to allow counting rates close to 1 million counts using a special data acquisition system¹⁷).

Figure 4

5.0 Acknowledgements

This work was supported by the Office of Health and Energy Resources (OHER) of the Department of Energy

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12/18/92