A Low-Angle Neutron Data Acquisition System for Molecular Biology

By B. P. SCHOENBORN, J. ALBERI,* A. M. SAXENA and J. FISCHER,* Biology Department, Brookhaven National Laboratory, Upton, New York 11973, USA

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The BNL low-angle spectrometer was designed and constructed to accommodate a variety of low- and medium-angle experiments in molecular biology. This spectrometer incorporates a 3He two-dimensional position-sensitive counter. Multilayers or conventional crystals are used as monochromators.

Introduction

The low-angle Brookhaven spectrometer system utilizing a two-dimensional position-sensitive counter was designed to accommodate a variety of experiments in molecular biology requiring good low-angle resolution. Biological structures requiring low-angle analysis techniques fall into two groups: (1) non-ordered systems, i.e. proteins or protein complexes in solution (Engelman & Moore, 1975; Schmatz, Springer & Schelten, 1974), and (2) ordered systems with large spacings like muscle, collagen, membranes, etc. (Worcester, Gillis, O'Brien & Ibel, 1975; Miller, Brodsky, Hulmes, Jenkins & White, 1975; Schoenborn, 1976).

For structural investigations into such systems, data are ideally needed to a low scattering angle of 0-2° at 4.5 Å or a minimum Q of 0.005 Å⁻¹ (Q = θ x 2π/λ). Depending on the type of structure, data often extend to the high-angle region, say 30°. Apart from the low-angle requirements, the spectrometer must have good resolution to resolve diffraction peaks from samples with crystal spacings up to 1000 Å or even larger. While it is desirable to build a spectrometer to such scattering conditions, given reactor conditions might not permit this and compromises have to be made between flux, resolution and smallest angle. The low-angle spectrometer described here was designed to be used at the High Flux Beam Reactor (HFBR) beam pipe working at ~4-2 Å or at the H4 satellite station working at 2-4 Å with an eventual move of this system to the cold moderator B beam at H9 (Fig. 1).

Spectrometer parameters

In order to assign general dimensions to such a spectrometer, the sample dimension, counter resolution, beam divergence and flux have to be considered. Biological samples are often very small since many cellular subfractions occur in very small amounts, which are difficult to extract and purify. For solution scattering, samples of ~0.1 cm³ are typical with protein concentration in the range of 5 to 30 mg cm⁻³. Since the samples are often dissolved in H₂O, D₂O or mixtures thereof, the sample thickness are of the order of a few millimeters for an attenuation of ~1/e. For solution scattering, a sample diameter of 6 mm was chosen with sample thickness of 2 to 6 mm depending on the sample's absorption. Such a sample size is also well matched to the counter resolution which is <3 mm.

Ideally, data are needed to zero scattering angle; in practice the smallest attainable angle is determined by wavelength, beam collimation and beam-stop characteristics. For most practical considerations, a compromise between flux and resolution has to be made to obtain acceptable counting statistics within a given time.

For the Brookhaven HFBR with a D₂O moderator, the low-energy neutron distribution is nearly Maxwellian, peaking at ~1-1 Å. Owing to the rapid decrease in flux with increasing wavelength, experiments with >5 Å are at present not feasible. The recent installation of the cold moderator will increase the usable wavelength range to at least 10 Å.

At present, the low-angle biology spectrometer is either used at 4-2 Å at a beam pipe (H4B) or at the H4S satellite station as shown in Fig. 2.

At H4S, monochromatic neutrons are selected from a pyrolytic graphite crystal set for 2.37 Å, a wavelength suitable for good λ/2 attenuation by a tuned graphite filter. The graphite filter is composed of eight stacked graphite plates with a combined thickness of 5 cm. The beam flux characteristics for λ and λ/2 were measured with an analyzer crystal and the absorption curves are shown in Fig. 3. With eight filters, the flux at λ/2 is less than 10⁻³ of the flux at λ = 2.37 Å. The setting of the filter is easily adjusted by rotation of the filter around two orthogonal axes perpendicular to the beam. This adjustment is done with an analyzer crystal set for λ/2. To test if the filter setting is correct in a configuration where the counter is set for low angles and is not movable to higher angles, the linear absorption coefficient of a set of absorbers is measured and compared to previously determined absorption coefficients for λ and λ/2. The percentage λ/2 contamination is then readily calculated.

The desired beam divergence with a beam diameter that is matched to the flat cylindrical solution sample cells is achieved by variable-length collimators made from a steel pipe with beam-defining discs spaced every 5 cm. These beam-defining discs have a 6 mm ⊙ circular hole and are made from cadmium fronted by a 2 mm thick boron carbide-lead absorber which is beveled toward the beam-defining edge of the cadmium disc.

For most solution scattering experiments, a 1.4 m collimator is used, yielding a flux at 2.37 Å of ~5 × 10⁷ n cm⁻² min⁻¹. The beam stops used in such experiments are 5 cm-long aluminum cylinders packed with fine-grain (200 mesh) boron carbide powder. Tests with boron carbide, boron polyethylene, 6Li metal, 7Li fluoride and 9Li carbonate showed that boron carbide gave less beam-stop scattering than the other materials.

Two-dimensional detector

This low-angle spectrometer uses a two-dimensional position-sensitive detector with an active area of 18 x 18 cm. This detector is a multiwire proportional counter using the
The $^3$He($n$,p)$^3$H reaction which has a reaction energy of 764 keV and a cross section of 5327 barns for thermal neutrons. The counter operates at about 3 kV with a gas filling of 6 atm $^3$He, 4 atm Ar, and 0.5 atm CO$_2$ or CH$_4$ with an efficiency close to 75% at 2.4 Å.

The neutron position is determined from the induced charge on two orthogonal grids of resistive wires by a charge-division technique. A detailed description of the prototype counter and read-out electronics is given by Alberi, Fischer, Radeka, Rogers & Schoenborn (1975), and a summary of the characteristics is given in Table 1. The only modification made to this counter over the last four years has been the addition of a getter purifier to reduce contamination of the highly purified gases.

Fig. 1. Beam and spectrometer layout at the Brookhaven high flux reactor with the biology low-angle instrument positioned at H4BP.

Fig. 2. Biology low-angle spectrometer layout at either H4BP or H4S.
Table 1. Two-dimensional position-sensitive detector characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>1. Neutron detection efficiency at 2.4 Å</td>
<td>75%</td>
</tr>
<tr>
<td>2. Position resolution at (V_p - V_c = 3000) V</td>
<td>2.7 mm</td>
</tr>
<tr>
<td>3. Positional uniformity of detection efficiency</td>
<td>3%</td>
</tr>
<tr>
<td>4. Integral linearity</td>
<td>1%</td>
</tr>
<tr>
<td>5. Differential linearity</td>
<td>0.1%/% of full scale</td>
</tr>
<tr>
<td>6. Event resolving time</td>
<td>1 s</td>
</tr>
<tr>
<td>7. Event processing time</td>
<td>3 s</td>
</tr>
<tr>
<td>8. (\gamma)-ray sensitivity ((^{60}\text{Co}))</td>
<td>(2.7 \times 10^{-6}) detected (\gamma)/incident (\gamma)</td>
</tr>
<tr>
<td>9. Sensitive area</td>
<td>18 × 18 cm</td>
</tr>
<tr>
<td>10. Gas-filling composition (absolute pressure)</td>
<td>(6) atm (^{3}\text{He}), (4) atm Ar, (0.5) atm (^{12}\text{CO}_2)</td>
</tr>
</tbody>
</table>

Monitoring of the pulse-height distribution does indicate some minute changes in the counting-gas composition requiring an occasional adjustment of the anode voltage to maintain reasonable pulse-height spectra and \(\gamma\)-ray discrimination. The main disadvantage of such high-pressure counters is caused by the required thick window of 9.5 mm aluminum with a resultant 7% beam attenuation by scattering.

A revised version of the chamber (20 × 20 cm) has recently been developed. It simplifies the construction, and improves the efficiency, spatial resolution and gas stability. The two dimensions are read out from one cathode and one anode. The anode also serves as a pulse-height monitor to discriminate against \(\gamma\)-rays. The anode-to-cathode gaps are 7.5 mm.

The cathode consists of thin metal strips spaced 5 mm on a glass plate. The strips are interconnected by resistors. Similarly the anode wires (25 \(\mu\)m \(\varnothing\), 2.5 mm spacing) are also interconnected by resistors (see Fig. 4).

The position of the interaction is read out from the ends of each resistor chain by a charge-division method.

The gas, \(6\) atm \(^{3}\text{He}\), \(4\) atm Ar and \(0.5\) atm \(^{12}\text{CH}_4\), is circulated with a small pump through a purifier removing harmful outgassing and decomposition products.

The position read-out accuracy is better than 0.3% of full scale for \(x\) and \(y\) and is completely linear as measured by a uniformly spaced (1 cm) raster scan. For routine linearity tests a calibration plate, with neutron-transparent concentric circular rings implanted into the absorber material of boron carbide mixed with epoxy, is mounted on the counter window and the counter is then exposed to neutrons incoherently scattered by a polyethylene sample. The spacings of the observed ring pattern are then used to calculate the grid parameters and to test for linearity.

The counting linearity of the detector was measured by a grid scan with a small, highly collimated beam. The counter showed a periodic counting-rate variation of 3% in the vertical direction. This gain variation is caused by a small fluctuation in the anode field due to the wire spacing of 2.54 mm. In most cases, no correction is made for this variation since data are integrated over a few cells and this gain change is averaged.

The computer system for the low-angle spectrometer is shown in Fig. 5. The active counter window is decoded into a 128 × 128 matrix with a data processing rate capacity > \(10^5\) counts s\(^{-1}\). A dead-time counting unit is incorporated but seldom used with the low scattering intensity from biological samples. The two-dimensional counter data are decoded into an external direct-access memory with a derandomizer input as described by Dimmler & Hardy (1976). This Fortran-programmable computer system will permit on-line data analysis like strip or radial integration, which are directly displayed. Final data processing is done on the CDC 7600 computing system with the data transferred via magnetic tape.

![Fig. 3. Beam intensity as a function of absorber thickness for \(\lambda\) and \(\lambda/2\) to determine the wavelength-dependent linear absorption coefficients of the absorber material (polyethylene).](image)

![Fig. 4. The new 2D position-sensitive counter showing the anode and cathode planes.](image)
Monochromators

In both presently used spectrometer configurations pyrolitic-graphite monochromators (Riste & Otnes, 1969) are used with a graphite filter for $\lambda/2$ removal at $\lambda = 2.37$ Å ($H_{4} S$) or a cooled Be filter at $\lambda > 4.0$ Å ($H_{4} B$); pyrolitic-graphite monochromators at these wavelengths are efficient (80% or better) but do have a large intrinsic beam divergence (~0.4°).

For operation at the cold moderator beam, multilayer monochromators (Schoenborn, Caspar & Kammerer, 1974) will be used at the end of a guide tube, Fig. 6. These devices are made by alternately depositing thin films of two materials with different scattering factors. Such a system is periodic in the direction normal to the plane of the multilayer with a Bragg spacing equal to the combined thickness of two films. If the two materials chosen have opposite scattering factors, like manganese and germanium, then multilayers with a small number of bilayers give rise to high reflectivities, small beam divergence and no high-order contamination (Saxena & Schoenborn, 1977a). If one of the repeating bilayers is a magnetic material like iron, then such multilayers can be used as efficient polarizers (Lynn, Kjems, Passell, Saxena & Schoenborn, 1976; Mezei, 1976). In practice, the Bragg spacing of such monochromators can be anywhere between 40 and 200 Å (or longer) with a selectable $\Delta \lambda/\lambda$ lying between 0.05 and 0.25 and an efficiency close to 100%. Multilayer monochromators offer, therefore, a number of advantages for low-angle work, particularly for experiments with long-wavelength neutrons.

Data collection

In a typical solution-scattering experiment, as in the determination of radii of gyration of a protein complex (Moore, Engelman & Schoenborn, 1974), the scattering from a number of samples has to be measured. Since the scattering from the minute amount of protein is very small, the scattering from the solvent, the sample cell and sample-independent background have to be measured, scaled and subtracted from the protein data. In a typical case, data from eight different samples are collected: three protein samples in buffers with different $D_{2}O$ concentration, samples of the three different buffers, a blocked beam and an empty sample cell. These samples are mounted in a temperature-controlled sample changer under computer control and exposed to neutrons. Data from the samples are collected for an appropriate monitor count of the order of one hour per sample, but less for the background data. So that statistically significant data can be accumulated, the samples are recycled until the appropriate exposure time has been achieved. This sample-cycling procedure averages long-term background and beam variations and possible sample deteriorations.

Sample-to-sample scaling is done with the help of direct beam-attenuation measurement, while protein concentration is determined by spectroscopic or other techniques. The protein’s activity is often measured before and after exposure to neutrons so that possible sample denaturation can be monitored. The data for the individual sample runs are added, scaled to unit monitor flux and radially integrated before background and solvent subtractions.

A set of radially integrated data sets is given in Fig. 7 for empty cell, blocked beam, $H_{2}O$ and $D_{2}O$ solutions without protein. The analysis of such low-angle data is not the subject of this paper and is treated by a number of authors, notably Stuhrmann (1973).

The “protein pair” measurements on ribosomes by Engelman & Moore (1974, 1975) have to date been the most successful application of low-angle neutron techniques to biological problems. The very weak signal of the deuterated protein pair within the density-matched solvent-ribosome entity demands special care in data collection and processing techniques. Fig. 8(a) shows the data set from recent measure-
ments of the S7–S9 protein separation in the 30S ribosomal subunit (Langer, Moore & Engelman, 1978). In this case, the interference function caused by the separation of the two deuterated subunits is obtained from two samples: (1) a mixture of equal amounts of 30S particles containing the two deuterated proteins and the same particle with no deuterated proteins, (2) a sample containing an equal amount of 30S particles with one or the other of the proteins deuterated. Hoppe (1973) has shown that in the above case, simple subtraction of the two data sets will yield directly the interference function associated with the separation of the two deuterated particles.

For lamellar and paracrystalline preparations, sample-cell constructions vary widely depending on the preparation techniques used to achieve good orientation. For diffraction experiments from membranes like lecithin bilayers, where orientation is achieved by spreading the lipid on a thin quartz slide, the sample is mounted on a goniometer within a temperature- and humidity-controlled aluminum chamber. Data are then collected by rocking these oriented specimens through the Bragg angles (Fig. 9). For correct integration of the reflections, the spectrometer characteristics, such as beam divergence and sample mosaic, have to be known in order to determine the size of the reflection as a function of Bragg angle. The accurate determination of the vertical extent of reflections is particularly important for the weak higher orders (Saxena & Schoenborn, 1977b). Background can then be determined from areas close to the reflections or can be integrated over the same counter region (2θ) but at a scattering angle before and after the respective Bragg angle. The latter background determination is more accurate, but can however only be used for samples with small mosaic width.

For structural analysis of ordered systems with a large mosaic like retinal rods (Yeager, 1975) or oriented purple membranes (King, Bogomolni, Hwang, Stoechenius & Schoenborn, 1977), the use of a 2D counter is particularly advantageous since many reflections occur simultaneously.
Data analysis in such cases where reflections often overlap is, however, more difficult. In the analysis of intact retinal rods, Yeager (1975) gave a detailed analysis of this problem. He obtained the background from the equatorial scattering by rotating the retina by 90° from the Bragg reflecting position. To fit a smooth curve to this background data he used polynomial functions with multiple linear-regression analysis. Peak integration was then achieved by fitting Gaussians to the background subtracted data.

The authors are indebted to many who contributed to the successful design, manufacturing and maintenance of the low-angle data-acquisition system; notably D. E. Engelman, S. Rankowitz, E. Caruso, T. Clifford, V. Radeka, G. Dimmler, P. Gileeny, L. Roger, M. Kelley and H. Okuno.

![Intensity contour map of a dipalmitoyl lecithin sample](image)

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**References**


**The Converging-Beam Small-Angle Neutron Diffractometer: Calculated Resolution**

By A. C. Nunes, Department of Physics, University of Rhode Island, Kingston, Rhode Island 02881, USA

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The resolution function of a converging-beam system for use in a small-angle neutron diffractometer is investigated. It is shown that beam convergence in itself has only a small effect on resolution. Also a consideration is the monochromator and the correlation between wavelength and angle which it imposes upon the beam falling on the sample. Only minor modifications of existing monochromator and shield are required to produce a converging beam.

**I. Introduction**

Small-Angle Neutron Scattering (SANS) is finding application in an increasing number of disciplines including physics, chemistry, biology and applied science (Schmatz, Springer, Schelten & Ibel, 1974). There appear to be two reasons for...