Macromolecular Crystallography with Synchrotron Radiation. II. Results

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Abstract

Crystallographic data for three different protein crystals (glycogen phosphorylase b to 2 Å resolution, β-lactamase I to 2.5 Å resolution and troponin C to 6 Å resolution) have been recorded using the intense synchrotron radiation beam emitted by the DCI storage ring at LURE and the DORIS storage ring at DESY/EMBL. Reduction in exposure times of approximately 50-fold and an increase in crystal lifetime of at least fivefold are observed when data recorded at LURE are compared with those recorded with a conventional rotating-anode source. These factors have made possible data collection which otherwise would have been impossible. For large crystals of phosphorylase b a greater reduction in exposure time (× 125) is made possible by the focusing geometry of the synchrotron-monochromator system which allowed irradiation of a larger volume of the crystal (collimator size increased from 0.3 to 1.0 mm) without significant increase in spot overlap on the film. The data processing statistics for phosphorylase b and β-lactamase compare favourably with those from data recorded on a conventional source (improvements in merging R values of between 1 and 4%). For phosphorylase b, but not for β-lactamase or troponin C, significant thermal diffuse scatter is observed on photographs recorded with synchrotron radiation. The possible origin of this phenomenon and its effect on data processing are discussed.

I. Introduction

This paper describes our observations in the use of synchrotron radiation (SR) for the measurement of X-ray diffraction data from protein single crystals. In the studies we have exploited the high spectral brilliance of this source: after monochromatization, an intense and sharply collimated beam is obtained throughout the most useful wavelength range (1-2.5 Å) and especially around the standard 1.54 Å wavelength (Kahn, Fourme, Gadet, Janin, Dumas & André, 1982; Bartunik, Fourme & Phillips, 1982). In order to provide answers to certain questions regarding the practical advantages of SR in terms generally useful to the protein crystallographers, we have summarized the experimental results obtained at LURE for three proteins whose structures are currently under investigation at Oxford (Table 1).

The three proteins represent various problems often encountered in protein studies. For glycogen phosphorylase b the crystals are large and well ordered, but the size of the unit cell and the consequent weakness of the intensities at high angle makes data collection beyond 2.5 Å resolution impossible with a conventional X-ray source. For β-lactamase I only small crystals are available although the unit cell is also small. For troponin C, the crystals are small and the unit cell is large; collection of X-ray data even to 6 Å resolution is impracticable on a rotating-anode source.
Table 1. The three proteins studied at LURE

<table>
<thead>
<tr>
<th>Protein</th>
<th>Crystal form</th>
<th>Crystal size</th>
<th>Molecular weight of asymmetric unit</th>
<th>Number of 24 h shifts used</th>
<th>Data recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Rabbit muscle phosphorylase b</td>
<td>P432 2 2</td>
<td>2 x 1 x 1 mm</td>
<td>100 000</td>
<td>8</td>
<td>Native and mercury-derivative data to 2.0 Å</td>
</tr>
<tr>
<td>2. Bacillus Cereus β-lactamase I</td>
<td>C2</td>
<td>0.3 x 0.1 x 0.03 mm</td>
<td>28 000</td>
<td>11</td>
<td>Native data to 2.5 Å, samarium- and platinum-derivative data to 2.5 Å</td>
</tr>
<tr>
<td>3. Rabbit muscle troponin C</td>
<td>I4, or I4 2</td>
<td>0.3 x 0.1 x 0.1 mm</td>
<td>140 000</td>
<td>1</td>
<td>20% of the native data to 6.0 Å</td>
</tr>
</tbody>
</table>

In addition, the paper describes the collection of a set of 3 Å data for glycogen phosphorylase b using the synchrotron radiation source at DESY, the European Molecular Biology Laboratory, Hamburg. Although it is not our purpose to provide a detailed comparison between synchrotron sources, these data are discussed briefly because they are included in the final merged set for phosphorylase b.

II. Experimental methods

Paper I in this series (Kahn et al., 1982) gives details of the radiation source and the storage ring DORIS, and describes the polarization of the beam and the correction required for X-ray diffraction data. The paper also provides information on the set-up used on the protein crystallography beam line with a bent-germanium-crystal monochromator providing a convergent beam of tunable wavelength. With the source operating at 1.72 GeV and a typical average current of 120 mA, the number of photons s⁻¹ mm⁻² behind a 0.3 mm diameter collimator is approximately 2 x 10¹⁰ at 1.54 Å wavelength (Lemonnier, Fourme, Rousseaux & Kahn, 1978). The double-focusing X-ray camera used for protein crystallography at DORIS/EMBL is described by Bartunik et al. (1982). The flux at the sample with DORIS operating at 4.75 GeV and 15 mA is 5 x higher at the same wavelength than at LURE.

The experiments below were carried out at 1.54 Å (phosphorylase b) or 1.4 Å (β-lactamase I and troponin C). The powder diffraction pattern from an aluminium wire was used to calibrate the wavelength. The resolution of the monochromator Δλ/λ has a minimum value of 0.002 at λ = 1.4 Å and rises to a value of 0.003 at λ = 1.54 Å.

At LURE, all data were collected on an Enraf–Nonius Arndt–Wonacott oscillation camera (Arndt, Champness, Phizackerley & Wonacott, 1973) in which the standard control unit had been modified so as to provide repeated oscillations for short exposure times. At EMBL, data were recorded using a modified Arndt–Wonacott oscillation camera (Bartunik, Clout & Robrah, 1981). The beam intensity is monitored continuously with an ionization chamber and the timing of each step of the oscillation adjusted accordingly with a PDP 11/10 computer. Conventional flat-plate cassettes using three film packs were used for recording β-lactamase I and troponin C data. For phosphorylase b, a crystal-to-film distance of 96 mm is required in order to resolve adjacent reciprocal-lattice spots on the film and at this distance the flat-plate cassettes (recording area circle diameter 120 mm) are too small to record data beyond 2.5 Å resolution. Hence, in order to avoid spot expansion cylindrical cassettes were used.

The cylindrical cassettes (radius 96 mm) were designed by Dr D. G. R. Yeates and built in the laboratory workshop by Mr M. G. Pickford. The cassettes (four in all) were mounted on the carousel with cylindrical axis parallel to the oscillation axis. For the zeroth reciprocal-lattice level normal to the oscillation axis, the diffracted beams are everywhere normal to the film. For other levels, the expansion of the spot in the direction parallel to the oscillation axis is proportional to sec ζ, where ζ is the angle between the generator for the nth layer line and the normal to the rotation axis. Correction for oblique incidence is therefore only required in this direction. The arc length of the cassette was 160 mm which allowed data to be recorded to values 2θmax = 47.7° (dmin = 1.9 Å for λ = 1.54 Å). The length of the cassette parallel to the cylindrical axis was 114 mm corresponding to 2θmax = 30.6° (dmin = 2.9 Å for λ = 1.54 Å) but since this region corresponds to the blind region for oscillation geometry, these data were recollected using a crystal mounted about a different axis. The film holder was designed to hold two 175 x 125 mm films. In retrospect it would have been preferable to use three film packs with the intense synchrotron source in order to...
Table 2. Exposure times and crystal lifetimes of the proteins studied at LURE and on a conventional source

<table>
<thead>
<tr>
<th>Protein</th>
<th>Oscillation angle</th>
<th>Resolution</th>
<th>Exposure time on GX6</th>
<th>Exposure time at LURE</th>
<th>Reduction in exposure time at LURE</th>
<th>Exposure per crystal on GX6</th>
<th>Exposure per crystal at LURE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphorylase b</td>
<td>0.5°</td>
<td>20 Å</td>
<td>13 h</td>
<td>1020 s × 46</td>
<td>375 s* × 125</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>β-Lactamase I</td>
<td>2.0°</td>
<td>25 Å</td>
<td>16 h</td>
<td>1500 s × 38</td>
<td></td>
<td>5</td>
<td>up to 16†</td>
</tr>
<tr>
<td>Troponin C</td>
<td>2.5°</td>
<td>60 Å</td>
<td>16 h</td>
<td>1250 s × 46</td>
<td></td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

*The first figure refers to 0.3 mm collimator and the second to 1 mm collimator (see text).
†The lifetime of the β-lactamase I crystals is not governed by simple radiation damage on the SR source, as described in the text.

record the whole range of intensities with one exposure. With a two-film pack about 50% of the low-angle data (below 5 Å resolution) were too intense to measure when exposures appropriate for recording the 2 Å data were used.

The diffraction experiments at LURE (Table 1) were carried out during 24 h shifts in the months between October 78 and October 79. During these shifts the storage ring was dedicated to the production of SR. After injection of electrons into the storage ring and subsequent energy ramping, the electrons circulate in the ring emitting useful SR. The electron current subsequently decays at variable rates. After injection the current was generally around 200 mA, and re-injection was carried out when it dropped below about 80 mA. The time between injections varied between 2 and 12 h, with an average of about 7 h. A visual display of the beam current allowed the user to adjust by hand the exposure times accordingly. The camera usually did not require realignment after a new injection of electrons.

The experiments at DORIS/EMBL were carried out during 9 d of 'parasitic' beam time in November 1979 when DORIS was operating in single-bunch mode at 4.75 GeV and about 15 mA with approximately 1 h between injections.

All experiments described for the conventional source in Oxford were carried out on an Enraf-Nonius Arndt-Wonacott oscillation camera mounted on an Elliott GX6 rotating anode, run at a tube power of 40 kV and 40 mA, and with nickel-filtered or graphite-monochromated CuKα radiation. The collimated beam was divergent.

The strategies used for the data processing of the phosphorylase and β-lactamase data depended upon the computing and densitometer facilities available at the time. The two approaches are slightly different and are described separately.

III. Results

III.1. Phosphorylase b

Recording conditions. Although large crystals can be grown routinely, phosphorylase b presents problems for data collection because of its large subunit (and asymmetric unit) molecular weight of 97 333. The crystal structure of glycogen phosphorylase b has been solved at 3 Å resolution (Weber, Johnson, Wilson, Wild, Yeates & Jenkins, 1978).

Collection of data to 2 Å resolution was begun in Oxford. A 0.5° oscillation photograph with data extending to 2 Å required 13 h exposure with a 0.3 mm diameter collimator, the beam being smaller than the crystal in all dimensions. A 0.5° oscillation photograph at LURE with 1.54 Å wavelength required an exposure time of 17 min using the same diameter collimator. This represents an improvement in exposure time of 46 × for SR. A greater gain proved possible with the large (0.8 x 0.8 x 1.5 mm) phosphorylase b crystals because of the convergent geometry of the beam from the bent-Ge-crystal monochromator at LURE. The 0.5° oscillation angle was selected as a compromise which allowed the largest oscillation angle with minimal number of overlapping reflections and least background scatter on the film. The convergent beam, which significantly reduced the spot size for a given collimator, allowed us to increase the collimator diameter to 1.0 mm (and hence spot size) without increasing significantly the proportion of overlaps. This resulted in a larger crystal volume being irradiated and exposure times now dropped to 6:25 min on average, a reduction of 125 × compared with the conventional source. These results are summarized in Table 2.

Oscillation photographs recorded at LURE and in Oxford are reproduced in Fig. 1. It is clear that the intensities of the high-angle data are stronger on the synchrotron photo. In fact the 13 h exposure with the conventional source is sufficient to damage the crystal severely, leading to a loss of intensity of the high-angle data.

Although quantitative estimates of radiation damage are difficult because individual crystals vary greatly, we have noted at least a fivefold improvement in effective crystal lifetime using SR. With a limit of 16 h exposure on the rotating anode before the onset of unacceptable radiation damage, only one 0.5° oscillation photograph with data extending to 2.5 Å could be recorded from each crystal. At LURE, the
high-resolution (2 Å) data disappeared in general after 1.5 h (total exposure, including orientation photographs) with a 0.3 mm collimator which permitted five 17 min 0.5° oscillation photographs. With the larger (1 mm collimator) an average of ten photographs per crystal (variation 4–19 photographs per crystal) could be obtained before radiation damage became unacceptable. This improvement in essence made possible collection on phosphorylase b to 2 Å, as the oscillation method is much more effective if more than one exposure can be recorded per crystal, so that partially recorded terms may be summed.

From Fig. 1 it can be seen that considerable subsidiary scattering occurs from crystals of phosphorylase b. A ghost lattice of diffuse spots is visible on both photographs but is much more apparent on the photograph recorded with synchrotron radiation. This diffuse scattering varied from crystal to crystal but was always dramatically more apparent on photographs recorded with synchrotron radiation.

Data collection and processing. Crystals of phosphorylase b form tetragonal prisms, elongated in the c(c*) direction and are easily mounted to rotate about this axis. 2 Å data over the range $\varphi = 0$ to $45^\circ$ were collected in a series of 0.5° oscillation photographs using 11 crystals and an overlap of at least 0.5° between crystals. Most reflections were therefore measured four times, twice as Friedel equivalents ($hkl$ and $hkl$) on the same film and twice as symmetry-related equivalents on a different film. Data in the blind region were collected by adjusting a crystal to rotate about $a^*$ by means of a simple yoke attached to the goniometer head. An incomplete data set in the range $\varphi = 0$ to $30^\circ$ was recorded using three crystals.

Data to 3 Å resolution were recorded using a conventional source as described previously (Wilson & Yeates, 1978; Weber et al., 1978). As part of a series of metabolite binding experiments, data to 3 Å resolution for a native crystal were also recorded at the DESY Synchrotron Source, Hamburg. At 4.75 GeV 15 mA, exposure times of 50–70 s deg$^{-1}$ were required for data to 3 Å resolution with a wavelength of 1.3 Å. This compares with exposures of 7200 s deg$^{-1}$ with a rotating-anode source (wavelength 1.5418 Å). Data in the range $\varphi = 0$ to $45^\circ$ were collected in steps of 1° oscillations from one crystal which was moved twice during the experiment to expose three different areas. The crystal-to-film distance was 113 mm. Data collection was completed within a period of 2.5 h. As at LURE the reduction in exposure times is significant (100 x comparing data with $\lambda = 1.3$ Å with those recorded with $\lambda = 1.54$ Å), radiation damage is less, and diffuse scatter is more pronounced.

The data were processed using the programs OSCAR, FILMPACK and SSM (Wilson & Yeates, 1979), modified to take into account the cylindrical geometry of the recording cassettes and the polarization of the synchrotron beam. Setting still photographs (30 s exposure) were recorded with flat-plate cassettes at $\varphi = 0$ and 90° for $c^*$ data and $\varphi = 0$ and $+42^\circ$ for $a^*$ data. These were used to determine the crystal orientation matrix, crystal-to-film distance and unit-cell parameters. The correctness of this matrix was checked by comparing the data photographs with colour-coded plots of the reflections produced by the computer. In some instances small corrections were applied to the matrix to obtain satisfactory agreement.

The reflecting range of the crystal ($\gamma$) was found empirically from comparison of different plots to be 0–25° at LURE and 0–35° at DESY. The data films were digitized on an Optronics Photoscan P1000 densitometer using a 100 μm raster and 100 μm spot size. The size of the reflections on the film ranged from

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**Fig. 1. Oscillation photographs for phosphorylase b.** The upper photograph (a) was recorded in Oxford on an Elliot GX6 rotating anode and required an exposure of 13 h. The lower photograph (b) was recorded at LURE with an exposure time of 6 min. The films were exposed using a cylindrical cassette and the data extend to 2 Å at the edge of the film. Further details and discussion are provided in the text.
9 × 9 to 11 × 11 raster points. Film orientation matrices were determined from the positions of the fiducial spots and refined against the positions of 40–50 strong reflections. Optical density values were corrected with reference to an empirical curve for non-linearity in the response of the film and densitometer. Reflections were integrated and corrected for background as described previously (Wilson & Yeates, 1979) and those reflections whose optical density values exceeded the saturation level of the film or whose backgrounds differed by more than 10% from the mean background were rejected. A typical 2 Å film contained some 2700 successfully integrated reflections including partials) and some 450 reflections rejected because of overlapping spots, some ten reflections rejected because of background inequalities and some 120 reflections which were too strong to measure on the weak film.

The mean deviation of the crystal-to-film distance from its ideal value of 96 mm was found to be 0·21 mm with a maximum deviation for one crystal of 0·59 mm. Such small differences between the position of the crystal and the cylindrical axis of the cassette do not interfere with the advantages of cylindrical geometry (diffracted beams nearly normal to the film) and nor do they interfere with spot prediction since it is the observed crystal-to-film distance that is used.

The weak and strong films were scaled together using the program FILMPACK and the intensities corrected for oblique-incidence absorption, and Lorentz and polarization effects. The expression for polarization was that given by Kahn et al. (1982).

\[ P = P_0(1 - \tau' \alpha), \]

where \( P_0 = (1 + \cos^2 2\theta)/2 \) and is the polarization correction for a reflection of a non-polarized incident beam with Bragg angle \( \theta \) from the specimen crystal.

\[ \alpha = \frac{\sin^2 2\theta \cos 2\psi}{1 + \cos^2 2\theta}, \]

where \( \psi \) is the angle between the projection of the diffracted beam and the horizontal axis of the synchrotron.

\[ \tau' = \frac{(I_0' - I_\alpha')}{(I_0' + I_\alpha')} = 0.89 \]

and is a measure of the polarization of the synchrotron beam at the height of the crystal. \( I_0 \) and \( I_\alpha \) are the horizontal and vertical components of the intensity of the incident beam after monochromatization by the germanium crystal. The vertical focusing by mirrors used on the double-focusing camera at DORIS/EMBL results in a broader distribution in the polarization of the beam incident on the sample since the effective vertical component is increased. At the same time the average polarization is decreased to a typical value of \( \tau' \sim 0.6 \). It has been found, however, that such a difference in \( \tau' \) has practically no influence on the data to 3 Å resolution. No radiation damage or absorption corrections were applied. For 50 two-film packs (2 Å e* data) the average merging \( R \) (defined as in Table 3) for intensities of equivalent reflections within the strong film was 0·076 and within the weak film was 0·066. Approximately 10% of the reflections had intensities which were measured as negative for either a fully recorded or partially recorded spot. The number of reflections rejected because they differed from their mean by more than four standard deviations represented some 0·20% of the data for the crystal mounted about e* and 0·24% of the data for the crystal mounted about a*.

The data films were then scaled together (Fox & Holmes, 1966) using the program SSM which determines relative scales and temperature factors for each film. Because of the tetragonal symmetry of the phosphorylase crystals, this procedure partially compensates for residual effects of radiation damage, crystal disorder and those absorption effects which are solely \( \phi \) dependent. Reflections partially recorded on two adjacent films were summed and equivalent reflections merged. The results are summarized in Table 3, and compared with the data to 3 Å resolution. The quality of 2 Å SR data as judged from the agreement between equivalent reflections is good and compares well with the 3 Å data set despite the difficulties associated with recording weak high-angle data. The merging \( R \) for the 3 Å DORIS/EMBL data set (0·076) compares well with the merging \( R \) for the 3 Å conventional source set (0·089).

There is a noticeable discrepancy between the intensities of equivalent reflections that have been fully recorded on one film and those partially recorded on two films (Table 3). The fully recorded intensities are systematically less than the partially recorded sums (Fig. 2). Similar trends have been observed by other workers (e.g. Irwin, Nyborg, Reid & Blow, 1976; Schmid, Weaver, Holmes, Grütter, Ohlendorf, Reynolds, Remington & Matthews, 1981). Although for phosphorylase data the differences are less than the standard deviations of the measurements for most reflections and rise to two standard deviations for strong reflections, the systematic nature of the trend gives cause for concern and is discussed later.

The data were passed through the program TRUNCATE (French & Wilson, 1978) which employs the principles of Bayesian statistics to correct negative and small intensities. The two 3 Å e* data sets were scaled to the 2 Å e* data using the program ANSC in which a local scale as a function of \( \phi \) was applied using values interpolated at 5° intervals. This scaling removed any systematic variations of scale with \( h, k, l \) or \( 4 \sin^2 \theta/\lambda^2 \). The three e* data sets were merged (program MERGE) to produce a final e* data set in
Table 3. Results of data processing for glycogen phosphorylase b

<table>
<thead>
<tr>
<th>Source</th>
<th>Data set</th>
<th>Resolution</th>
<th>Total reflections included</th>
<th>Number of independent reflections</th>
<th>Number† rejected</th>
<th>R_m †</th>
<th>Average intensity after scaling to strongest film (in data set) (arbitrary scale)</th>
<th>Number of negatives</th>
<th>Percentage of data with intensity &gt; 2σ</th>
<th>Overestimation§ of partials</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR (LURE)</td>
<td>c*mount</td>
<td>2 Å</td>
<td>119 827</td>
<td>45 003</td>
<td>390</td>
<td>0.106</td>
<td>5257</td>
<td>3436</td>
<td>74%</td>
<td>100% (2 Å)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>75% (3 Å)</td>
</tr>
<tr>
<td>SR (LURE)</td>
<td>a*mount ‡</td>
<td>2 Å</td>
<td>54 971</td>
<td>26 444</td>
<td>92</td>
<td>0.115</td>
<td>3600</td>
<td>1624</td>
<td>71%</td>
<td>49% (2 Å)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24% (3 Å)</td>
</tr>
<tr>
<td>SR (DESY)</td>
<td>c*mount</td>
<td>3 Å</td>
<td>53 125</td>
<td>18 487</td>
<td>136</td>
<td>0.076</td>
<td>2598</td>
<td>693</td>
<td>96%</td>
<td>9.6% (3 Å)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>not recorded</td>
</tr>
<tr>
<td>GX6</td>
<td>c*mount</td>
<td>3 Å</td>
<td>67 192</td>
<td>18 447</td>
<td>94</td>
<td>0.089</td>
<td>1576</td>
<td>741</td>
<td>74%</td>
<td>3.6% (3 Å)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>not recorded</td>
</tr>
</tbody>
</table>

† Reflections are rejected if |I_h - I_i| > 4σ.

‡ \( R_m = \frac{\sum \sum |I_h - I_i|}{\sum \sum I_i} \).

where \( I_i \) is the \( i \)th estimate of the intensity and \( I \) the mean intensity of reflection \( h \). All reflections are included, even those for which the intensity is fortuitously negative.

§ Overestimation defined as \( \frac{\sum (I_{π} - I_{φ})}{\sum \frac{1}{2}(I_{φ} + I_{π})} \).  

where \( I_{π} \) is the resulting intensity for the sum of two partially recorded intensities and \( I_{φ} \) the corresponding fully recorded intensity for reflection \( h \).

‡ These data also included data to 3 Å resolution recorded from eight precession photographs, \( h00, h11→h77 \) using GX6 rotating-anode source.
Table 4. Scaling and merging of phosphorylase b data sets

<table>
<thead>
<tr>
<th>Source/data set</th>
<th>Fractional change in F</th>
<th>Number of common reflections</th>
<th>Number of reflections measured more than once</th>
<th>Number rejected</th>
<th>Total number of reflections output</th>
<th>Name of merged data set</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR (LURE) c* 2 Å</td>
<td>0.0878</td>
<td>16 046</td>
<td>18 710</td>
<td>332 (0.7%)</td>
<td>47 282</td>
<td>c* 2 Å/3 Å merged</td>
</tr>
<tr>
<td>SR (DESY) e* 3 Å</td>
<td>0.0910</td>
<td>15 363</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GX6 e* 3 Å</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>e* 2 Å/3 Å merged set</td>
<td>0.0998</td>
<td>16 609</td>
<td>16 535</td>
<td>74 (0.1%)</td>
<td>56 780</td>
<td>Final 2 Å data set</td>
</tr>
<tr>
<td>SR (LURE) a* 2 Å</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

which the structure factors were the weighted mean when more than one observation was present. The 2 Å a* data set was then scaled and merged with the final c* data set in the same way to produce a final 2 Å data set containing some 56 780 reflections. The results are shown in Table 4.

III.2. β-lactamase I

Recording conditions. Crystals have been obtained for β-lactamase I from Bacillus Cereus 569 (Aschaffenburg, Phillips, Sutton, Baldwin, Kiener & Waley, 1978). The crystal form is summarized in Table 1. Individual crystals are generally no larger than 0.3 × 0.1 × 0.03 mm and the thinness of the plate-like crystals leads to weak diffraction. In experiments carried out at Oxford and LURE, a 0.3 mm diameter collimator was used so that the crystal was smaller than the beam in each dimension. A 2° oscillation photograph with data extending to 2.5 Å requires 16 h exposure on the rotating-anode GX6 source. At LURE the time for such an exposure dropped to 0.42 h, when 1.4 Å wavelength X-rays were used; an approximately 38-fold reduction.

Initial experiments at LURE indicated that the crystals were ordered to a resolution of at least 1.8 Å. However, because of the very small size of the crystals and the geometry of the X-ray source, the diffraction spots recorded were exceedingly small (size ~0.3 × 0.2 mm). As a consequence it was anticipated that these data would suffer from serious errors due to the ‘Wooster effect’ (Wooster, 1964), even when scanned on the finest practicable raster of 50 μm. It was also observed that after about an hour of exposure, data rarely extended beyond 2.5 Å resolution. Thus in order to exploit the efficiency of the screenless photographic method, and taking account of spot size and exposure times, we decided that the optimum strategy was the collection of 2.5 Å resolution data.

In eleven 24 h shifts at LURE we have recorded almost all of the unique data for the native protein, 97% for the samarium derivative, and 70% of the data for a platinum derivative. Two different batches of crystals were used in data collection. The first batch contained very small crystals that could only be irradiated for a short period of time before the crystals fragmented and the spots on the photographs appeared split. This was probably a result of the heating effect of the SR beam; distillation of liquid from the local environment of the crystal caused the fragile plate to collapse into the curve of the capillary tube. The use of the thicker, more robust, crystals from the
second batch (thickness increasing from 0.02 to 0.03 mm) partially resolved this problem, and a further improvement was gained by blowing air at room temperature over the capillary during collection of data. With these two improvements crystal lifetime increased from 2 h to over 12 h of irradiation by SR. The limitation on the amount of data collected from each individual crystal was now conventional radiation damage (that is loss of the higher-resolution data on subsequent films).

Photographs recorded in Oxford and at LURE are reproduced in Fig. 3. The convergent geometry of the X-ray beam at LURE produces much sharper reflections on the film, thereby decreasing the reflecting range of the crystal from 0.4° (Cu Kα radiation) to 0.3°.

The films were digitized using a Scandig III microdensitometer with a 50 μm spot and raster. Data processing was carried out on a PDP 11/70, using modified versions of the programs described by Stuart, Levine, Muirhead & Stammers (1979). These programs will be described in detail elsewhere. The most pertinent modifications are summarized here.

(i) In order to improve the estimation of the crystal orientation matrix a very simple 'post refinement' method was used; this method was initially suggested by P. Evans and developed by A. Wonacott (A. Wonacott, personal communication) and is based on the analysis of the relative intensities of partially recorded reflections measured on adjacent films.

(ii) In order to improve the estimation of the background level at each optical density point in the spot, the data were measured in two passes. On the first pass each individual optical density value in the area chosen for background estimation around the spot contributes to a set of normal equations which are then solved to give the least-squares best plane describing the background level in that region. On the second pass any points in poor agreement with this plane are rejected and the equation of the plane recalculated. An estimate of the standard deviation in the background measurement is derived from the mean deviation of the observed OD values from this plane. This standard deviation may be easily extended to describe the standard deviation in the spot intensity. Before accepting the background estimate a series of checks are carried out to ensure its reliability. These procedures are derived in part from those of Rossmann (1979).

(iii) A profile is determined empirically from the observed spot shape on each film and this is used to determine spot intensities as described by Rossmann (1979). However, whereas Rossmann determines each profile as a position-dependent average we allow the program to 'learn' the profile as it measures the data to allow for the very considerable change in spot shape in different areas of the film that are frequently observed with data for β-lactamase I.

(iv) Since the spots were very small (perhaps 0.7 x 0.35 mm on average) it was important that the predicted spot position should correspond precisely to the true position. In order to allow for any film distortions not easily described analytically the program allowed the spot windows to be displaced slightly from their expected position; for strong spots the window was moved towards the observed centroid of the OD measurements while for weaker spots a shift vector learnt from the stronger spots nearby was applied. Spots too far from the observed position were rejected.
Table 5. Results of data processing for β-lactamase I

<table>
<thead>
<tr>
<th>Source</th>
<th>Data set</th>
<th>Resolution</th>
<th>Total reflections included (number of partials)</th>
<th>Number of independent reflections</th>
<th>$R_{F_{ac}}$ (full)</th>
<th>$R_{F_{ac}}$ (full and partial)</th>
<th>Average F on arbitrary scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR (LURE)</td>
<td>b* native segment 1 (crystals 55,57)</td>
<td>2.5 Å</td>
<td>13 772 (7834)</td>
<td>4277</td>
<td>0.084</td>
<td>0.077</td>
<td>41.58</td>
</tr>
<tr>
<td>SR (LURE)</td>
<td>b* native segment 3 (crystals 45,48,49,50)</td>
<td>2.5 Å</td>
<td>9780 (4779)</td>
<td>3767</td>
<td>0.075</td>
<td>0.088</td>
<td>19.80</td>
</tr>
<tr>
<td>SR (LURE)</td>
<td>c* native segment 3 (crystal 61)</td>
<td>2.5 Å</td>
<td>3038 (1530)</td>
<td>1705</td>
<td>0.039</td>
<td>0.042</td>
<td>50.20</td>
</tr>
<tr>
<td>SR (LURE)</td>
<td>Amalgamation of b* data</td>
<td>2.5 Å</td>
<td>8044</td>
<td>7066</td>
<td>0.112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR (LURE)</td>
<td>Amalgamation of b* and c* data</td>
<td>2.5 Å</td>
<td>9749</td>
<td>7490</td>
<td>0.149</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SR (LURE)</td>
<td>Samarium segment 1 (crystals 54,64A)</td>
<td>2.5 Å</td>
<td>10 953 (4991)</td>
<td>3572</td>
<td>0.074</td>
<td>0.078</td>
<td>31.04</td>
</tr>
<tr>
<td>SR (LURE)</td>
<td>Samarium segment 1 crystals 52,38,64B</td>
<td>2.5 Å</td>
<td>14 823 (5229)</td>
<td>7398</td>
<td>0.121</td>
<td>0.118</td>
<td>34.10</td>
</tr>
<tr>
<td>GX6</td>
<td>Native (crystals 73,75)</td>
<td>3.0 Å</td>
<td>13 551 (5492)</td>
<td>4413</td>
<td>0.117</td>
<td>0.122</td>
<td>9.56</td>
</tr>
<tr>
<td>GX6/ SR (LURE)</td>
<td>Native GX6</td>
<td>2.5 Å</td>
<td>14 161</td>
<td>8286</td>
<td>0.135</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$R_{F_{ac}}$ (full) and $R_{F_{ac}}$ (full + partial) = \( \frac{\sum \sum |I_a - I_b|}{\sum I_a} \),

where the summation is over all reflections which are fully recorded only and those which are fully recorded are derived from the sum of two partially recorded respectively.

In total seven crystals were used in the collection of the native data set. If we assume these crystals are perfectly isomorphous with each other then we may use the accurately refined cell parameters as obtained from the rotation photographs from each crystal to estimate the long-term stability and reproducibility of the wavelength. Throughout these experiments the wavelength was set as accurately as possible to 1.40 Å. Our subsequent refinements indicate that the mean value was 1.404 Å with a r.m.s. variation of 0.007 Å. The experimentally observed resolution of the monochromator at 1.4 Å resolution is approximately 0.002 Å (Lemonnier, Fourme, Rousseaux & Kahn, 1978). Some deviations from perfect isomorphism might be expected from the different β-lactamase crystals, and these variations will be included in our estimate of the r.m.s. deviation of the wavelength. We consider therefore that this observed deviation of 0.007 Å indicates good long-term stability of the system.

Films in each pack were scaled together and corrected for Lp effects in the usual way using the polarization correction described above with the appropriate angles determined directly from the position of the spots on the films.

The final stage of data reduction was the merging together of the data from separate film packs. Because of limitations of store size of the computer, the data were merged together in segments which were then amalgamated to give the final data set. Negative fully recorded and partially recorded reflections whose sums were less than zero were rejected. An indication of the quality of the data can be obtained from the overall merging R factor (Table 5). Although the data sets for the crystals mounted about b* and c* respectively agree well internally, crystals mounted about c* exhibit greater variation in absorption and their data merge less well with the b* data sets.

III.3. Troponin C

Crystals suitable for a 6 Å resolution X-ray analysis of rabbit muscle troponin C (TNC) have been obtained (Mercola, Bullard & Priest, 1975), Table 1. The relatively large unit-cell volume and the small size of the crystals (less than 0.1 mm along the a and b axes) limit the rate of data collection and the possible resolution on the conventional source.

At LURE we obtained a 48-fold reduction in exposure time in comparison with Oxford, Table 1. As for β-lactamase I, the collimator diameter (0.3 mm) was sufficiently large for the crystal to be totally immersed in the beam at all times.
On the GX6 only one photograph could be obtained from each crystal before the onset of unacceptable X-irradiation damage. Five or more such exposures could be recorded at LURE.

We did not observe any significant increase in the resolution of data which could be recorded at LURE. There appears to be an inherent disorder of the TNC crystals. The angular reflecting range is large ($\gamma = 0.65^\circ$) and this inherent disorder might also explain our inability to date to grow larger crystals. No diffuse scatter was observed.

In twelve hours of beam time at LURE we recorded almost 20% of the data from the native protein. Good crystals of chicken TNC have recently been obtained elsewhere (Strasburg, Greaser & Sundaralingam, 1980) and we do not intend to proceed with our study of the present crystals.

IV. Discussion

At the few synchrotron radiation facilities where a suitable set-up is available, many projects relevant to macromolecular crystallography are in progress. The inconvenience implied by the use of distant shared facilities is more than counterbalanced by significant advantages. Those advantages result from the combined effects of several factors.

Owing to the high spectral brilliance of the source the geometry of the monochromatized beam may be made optimal without sacrifice of intensity. The set-up at LURE delivers a beam which is convergent in the horizontal plane (angle of convergence $1.7 \text{ mrad}$) and nearly parallel in the vertical plane (angle of divergence $0.3 \text{ mrad}$), with a bandwidth of $2 - 3 \times 10^{-3}$ at $\lambda = 1.54 \text{ A}$; as a result, the order-to-order resolution and the signal-to-noise ratio on the detector are at the level of those for a conventional double-focusing mirror-monochromator instrument. In the case of phosphorylase $b$ this meant that a greater volume of molecules in the neighbourhood of the absorbed X-ray quantum is the production of an excited electron which may dissipate its energy either as heat, radiation, or by direct transfer to neighboring atoms. Any of these events could lead to a destruction of molecules in the neighbourhood of the absorbed quanta. Secondary effects occur over a larger time scale and sometimes have been observed to continue after the X-ray beam is switched off. These effects probably arise from formation of free radicals stimulated by the radiolytic products of water [see discussion in Blundell & Johnson (1976), p. 252]. These active products, primarily the solvated electron, the hydroxyl radical and the hydrogen atom, can diffuse readily through the aqueous channels in the crystals inflicting damage on molecules far removed from the site of primary absorption. It appears that the deleterious effects of these chain reactions may be minimized by the use of short exposures with intense SR.

Further prolongation of crystal lifetime in the X-ray beams may be achieved by cooling the crystal, although the magnitude of the effect depends on the protein crystal investigated. Even blowing air at room temperature over the capillary increased the lifetime of $\beta$-lactamase crystals and cooling to 273 K (in a later experiment at DORIS/EMBL) increased the lifetime of phosphorylase $b$ crystals by a factor of 2–3. Low-temperature systems and their use have been described for conventional sources (e.g. Marsh & Petsko, 1973) and recently more sophisticated devices have been installed at synchrotron radiation facilities (Kahn et al., 1982; Bartunik & Schubert, 1982).

The continuous spectrum of the SR source was not used in our experiments. It is, however, clear that tunability is advantageous for high-resolution data collection as well as for anomalous dispersion studies.
Several arguments are in favour of the use of wavelengths shorter than the standard 1.54 Å radiation: (i) absorption corrections are reduced; (ii) data to higher resolution may be collected with a detector of fixed size, within the limits set by reflection overlaps; (iii) the signal-to-noise ratio is improved with less background scatter on the film. These advantages may well outweigh the disadvantage of decrease in scattering which is also proportional to \( \lambda^3 \). The data recorded for \( \beta \)-lactamase I at LURE (\( \lambda = 1.4 \) Å) and for phosphorylase b at DESY (\( \lambda = 1.3 \) Å) are encouraging.

**Diffuse scatter**

Considerable diffuse scattering is observed for the phosphorylase b photographs which is much more apparent on the synchrotron photos (Fig. I). Such effects are evidently dependent on the individual protein crystals for no diffuse scatter is observed with the \( \beta \)-lactamase I or troponin C crystals either with conventional or synchrotron sources. Thermal diffuse scattering arises when the atoms do not vibrate independently of one another but their vibrations are influenced by inter-atomic forces. The vibration modes of the lattice as a whole lead to subsidiary scattering.

Paraphrasing from James (1948, pp. 202–203), diffuse scatter is made up of independent contributions from each state of polarization of each lattice wave. A single such wave with wave vector \( \mathbf{g} \) will give rise to two maxima in the scattering distribution symmetrically disposed on either side, at distances \( \pm 2 \mathbf{g} \), of the reciprocal-lattice points. The intensities of these subsidiary peaks are much less than those corresponding to the main maxima. The actual motion of the lattice will be made up of a huge number of such lattice waves and to each of these corresponds a pair of maxima in the scattering function. Taken all together, these independent maxima will produce a continuous distribution about each reciprocal-lattice point. The shorter the lattice wave the greater will be the distance of the corresponding maxima from any given reciprocal-lattice point. Since the shortest acoustic waves in a crystal lattice have lengths of the order of twice the lattice spacing, the intensity distribution will be much more diffuse than that corresponding to the stationary lattice. Since one-phonon thermal diffuse scattering is much less sharply peaked than the main peak, then as the breadth of the instrumental profile or the width of the scan range is increased, relatively more diffuse scattering is included (Azároff, Kaplow, Keto, Weiss, Wilson & Young, 1974). In certain cases analysis of diffuse scatter can yield information on the shape and orientation of the molecule in the crystal (e.g. Amorós & Amorós, 1968). The existence of diffuse scatter poses problems for the correct estimation of intensity, especially for protein molecules where refinement of structures is only just beginning to become routine and quantitative. Various authors have reported widely varying amounts of thermal diffuse scattering (Azároff et al., 1974).

In the first instance we have integrated intensities as the basis of the reflecting range for the Bragg reflections and have ignored the diffuse scatter. The data appear satisfactory by the usual criterion with the possible exception of the partially recorded reflections which are discussed below. However, we have at present no estimate of the likely errors introduced into our data. Simple theory suggests that if the diffuse scatter is due to thermal effects rather than static disorder, the ratio of intensities of the diffuse spots to those of the sharp Bragg reflections should be linearly dependent on temperature. Cooling from 298 to 173 K therefore should show an enhancement of Bragg reflections over diffuse reflections by about 1.7. Such experiments are in hand for glycogen phosphorylase, and it is hoped will give some estimate of the errors. Strong temperature-dependent thermal diffuse scattering has been observed for some but not all proteins (Bartunik & Schubert, 1982).

One might speculate why the diffuse scattering is so much more apparent on synchrotron photographs for phosphorylase b. Comparison of photographs of similar intensities suggest that it is not simply a consequence of the intense synchrotron source, but that it may have become more apparent because of the focusing geometry used at SR sources. Disorder in the crystal lattice could also give rise to diffuse scattering effects and indeed some non-Bragg diffuse reflections can always be observed in photographs recorded with the continuous GX6 source (Fig. 1a). However, the diffuse scatter is considerably greater with the photographs recorded with SR. It has occurred to one of us (EAS) that the effect could be due to the pulsed nature of the synchrotron source. Pulses at LURE are typically 1 ns in duration with an interval of the order of 280 ns. Individual atoms in a protein molecule may vibrate on the ns time scale, as indicated by spectroscopic evidence (e.g. Lekowicks & Weber, 1980) but the times for vibrations of the molecule as a whole are likely to be considerably slower. For example, NMR evidence indicates that tyrosine rings in lysozyme flip between equivalent conformations at rates of the order of 10^4 s^{-1} (Campbell, Dobson & Williams, 1975). It is possible therefore that a resonance could occur between the pulsed radiation and the vibrating molecule. A fraction of the pulse energy could be dissipated within the crystal by promoting lattice vibrations, with those modes which can best make use of the periodic stimulation being most enhanced. In the phosphoryl-
ase b photographs for a crystal mounted about e* there is a tendency for the diffuse scattering to streak in the direction normal to the oscillation axis (Fig. 1), while for a crystal mounted about a* the diffuse scatter is streaked parallel to the oscillation axis. Such streaks indicate that the phosphorylase b molecules are vibrating in the plane perpendicular to the c axis of the crystal and this is consistent with the way in which the molecules are packed in the crystal lattice. Each subunit makes close contact with the other subunit of the physiologically active dimer at the twofold axis of symmetry at z = \( \frac{1}{2} \). The subunit spans the distance z = 0 to z = \( \frac{1}{2} \) and therefore also makes close contact with the adjacent molecule at z = 0. The contacts in the xy plane are less extensive (Johnson, Madsen, Mosley & Wilson, 1974).

There appears to be some correlation between diffuse scatter and time separation of pulses observed on photographs recorded at different synchrotron sources. Photographs recorded at the Synchrotron Radiation Source at Daresbury (Helliwell et al., 1981) (pulse time 0-12 ns separated by 2 ns) show less diffuse scatter than those recorded at LURE (pulse time 1 ns separated by 280 ns), which in turn show less diffuse scatter than those recorded at DESY (parasitic mode pulse time 0-15 ns separated by 1 μs). These data films were recorded with different crystals and with slightly different focusing geometries at the different synchrotron sources so a strict comparison is not possible. It is interesting to note, however, that photographs of native phosphorylase b recorded at DESY/EMBL in November, 1979 when the synchrotron usage was in parasitic mode (pulse time 0-15 ns separated by 1 μs) do show, on average, more dramatic diffuse scatter than those recorded in February 1981 (in a separate metabolite binding experiment not described here) when synchrotron usage was in main user mode (pulse time 0-15 ns separated by 2 ns). It might be anticipated that a pulse separation of 1 ns would be too small to stimulate thermal vibrations, while pulse times approaching 1 μs might be of the right order. While these observations must await quantitative verification, they provide preliminary support for a theory of diffuse scatter based on stimulated thermal vibrations.

**Partially recorded reflections**

The discrepancy between intensities of fully and partially recorded reflections for phosphorylase data gives some cause for concern. (No detailed analysis was carried out for the β-lactamase data.) Several factors may contribute.

(i) **Errors in crystal orientation matrices.** Experience has shown that small errors in crystal orientation matrices can lead to an underestimation of apparently fully recorded reflections. Some reflections may be flagged as fully recorded when in fact they are partially recorded. Orientation matrices for the 2 Å data films were obtained from setting stills recorded to only 3 Å resolution on flat plate cassettes. This was because of difficulties in marking fiducial spots accurately on the cylindrical cassette. More precise orientation matrices could be obtained by a post-refinement procedure (e.g. Schutt & Winkler, 1977; Rossmann, Leslie, Abdel-Meguid & Tsukihata, 1979). In our experience the discrepancy may be significantly reduced by extreme care in the derivation of the matrices by manual checks or by the use of post-refinement (results not shown). A good crystal orientation matrix also results in good quality data as judged by agreement between equivalent reflections. The fact that the \( R_m \) values for the SR 2 Å c* and 2 Å a* are roughly comparable (Table 3) suggests therefore that other factors may also contribute to the larger discrepancy between the fulls and the partials in the 2 Å c* data.

(ii) **Wooster effect** (Wooster, 1964). When a spot, which is small because it is partially recorded, is scanned with a 100 μm raster, errors in measurement may arise where there is a sharp variation in intensity. An average optical density would be obtained over an area that may include background and intense spot. However, since the sum of two logarithms is always greater than the logarithm of the average, these errors would lead to an underestimate of the partially recorded reflection.

(iii) **Diffuse scatter.** The data sets where the discrepancies between fully recorded and partially recorded intensities are greatest [the phosphorylase 2 Å c* (LURE) and the 3 Å c* (DESY)] are also those for which the diffuse scatter is greatest. The 2 Å a* (LURE) data have much less diffuse scatter than the 2 Å c* (LURE) data and here the agreement between fully and partially recorded intensities is good. While it is difficult to dissociate the effects of diffuse scatter from those arising from inadequacies of data processing, nevertheless it appears that diffuse scatter could provide a possible explanation. The reflecting range of a diffuse spot is greater than that of a Bragg reflection. For phosphorylase b, a 0-5° oscillation range was used. The angular reflecting width of the Bragg reflections was estimated to be 0-25°. If the reflecting width of the diffuse scatter is perhaps twice this value then for most fully recorded reflections the diffuse scatter will be partially recorded. For the partially recorded reflections, however, the sum of the two intensities will nearly always include all of the diffuse scatter (Fig. 4). Since diffuse scatter increases with \( \sin^2 \theta / \lambda^2 \) (James, 1948), this effect will become more pronounced at high angles. In order to overcome these problems it is possible that the dynamic mask procedure of Sjölin & Wlodawer (1981) may provide better estimates of intensities.

Clearly the agreement between partially and fully recorded reflections will depend upon the correct
choice of \( \gamma \), the reflecting range of the crystal. If \( \gamma \) is too small some reflections may be classified as fully recorded when in fact they are partially recorded and their intensities underestimated. On the other hand, if \( \gamma \) is too large extra noise will be included in the intensity estimates with no further improvement in agreement between fully and partially recorded. A test was carried out during the early stages of data processing on the native 3 Å data set collected on the GX6 rotating anode. The empirical estimate of \( \gamma \) as described in the data processing section was 0.35°. This was increased to 0.4° and the data reprocessed. There was no improvement in the discrepancy between fulls and partials. (Root-mean-square percentage difference between fulls and partials was 4.99% and 5.03% for \( \gamma = 0.35 \) and \( \gamma = 0.4 \)° respectively.) These results, and similar tests, suggest that our estimates of \( \gamma \) are reasonable. However, they neither prove nor disprove the proposals put forward in the previous paragraph because the reflecting range for the diffuse scatter is not known. Nevertheless, it seems plausible that, because the reflecting range of the diffuse scatter is greater than that of the Bragg reflections, more diffuse scatter will be included in the sum of two partials than in a reflection monitored as fully recorded.

Despite these reservations the phosphorylase b final 2 Å merged data set appears satisfactory and is being used to extend the resolution of the structure of glycogen phosphorylase b by constrained least-squares refinement. The results to date (which will be published elsewhere) appear promising. The data for \( \beta \)-lactamase 1, together with data from a further heavy-atom derivative, will be used shortly to compute a 2.5 Å resolution electron density map.

V. Conclusions

The main result of our experiences is that we have been able to collect three-dimensional data which were not in practice accessible with a rotating-anode X-ray source; SR is indeed better suited than any other available source to the collection of data close to the limiting resolution set by the intrinsic order of the crystal under study. This is especially true for crystals with large unit cells and/or small samples. SR is also a promising tool to study enzyme–substrate complexes stabilized at low temperature: (i) the high rate of data collection considerably shortens the necessary lifetime of the complex and the requirement of steady low temperature is similarly shortened; (ii) the use of SR, by increasing effective crystal lifetime, may reduce the number of crystals required in the study; this is ideal in such studies where it is wished to investigate the nature of a simple complex rather than a range of closely related ones. Finally, use of synchrotron radiation may lead to information concerning lattice vibrational modes through analysis of the diffuse scatter.

We wish to thank all members of the staff of the Laboratoire de l’Accélérateur Linéaire who operated the SR source at LURE and the staff at the EMBL for use of the DESY synchrotron. We thank the Medical Research Council of Great Britain for support, through post-doctoral fellowships (YSB and JAJ), and through post-graduate studentships (EAS and DLW), and the Science and Engineering Research Council for support through a post-doctoral fellowship (DIS). We thank the E. P. Abraham cephalosporin fund for support (RJT). We thank Professor D. Blow and Dr A. Wonacott for allowing us the use of the Imperial College Scandig microdensitometer in the early analysis of \( \beta \)-lactamase data. Finally, we are grateful to Professor Sir David Phillips for his continued encouragement in all of the projects described.

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