The emergence of the synchrotron Laue method for rapid data collection from protein crystals

By A. Cassetta[1]†, A. Deacon[1], C. Emmerich[1], J. Habash[1], J. R. Helliwell[1,2], S. McSweeney[2], E. Snell[1], A. W. Thompson[3] and S. Weisgerber[1]

[1] Department of Chemistry, University of Manchester, Manchester M13 9PL, U.K.
[2] SERC, Daresbury Laboratory, Daresbury, Warrington WA4 4AD, U.K.
[3] EMBL, Avenue des Martyrs, Grenoble Cedex, France

Synchrotron X-radiation (SR) is intense, polychromatic and collimated. It is widely exploited, in macromolecular crystallography, particularly using a monochromatized short wavelength beam. The spectral curve of SR, however, ideally lends itself to use of Laue geometry, i.e. the original diffraction experimental arrangement based on a stationary crystal and a polychromatic X-ray beam. Rapid exposure times and time-resolved crystallography studies, e.g. of enzymes, are now possible. Historical objections to the use of Laue diffraction data, particularly the multiplicity distribution, have been found not to be as limiting as once thought. The credentials of the Laue method have been established through a variety of Laue crystal structure analyses, involving photographic film as detector. Recently a three-dimensional arrangement of films, known as a toast-rack, has been used to alleviate problems with spatially overlapping spots. This paper provides a review of these results and then reports several developments. In particular, one of the first Laue analyses using an image plate as detector, namely of a cobalt substituted concanavalin A crystal, is discussed. Recent experimental developments, also at the Daresbury synchrotron, are then described. First, a large toast-rack has been used to record Laue data from a protein crystal. Secondly, a transmission X-ray mirror has been constructed from thin mylar (1.5 µm) and used to provide a λ_{max} filter instead of using aluminium foils. Thirdly, since the Laue method suffers from poor sampling of the low resolution data, a new method (known as LOT) has been introduced.

1. Introduction

The original X-ray diffraction experiment using a single crystal was based on an idea of von Laue and conducted by Friedrich and Knipping (Friedrich *et al.* 1912). It earned von Laue the Nobel Prize for physics in 1914. The basis of the idea was that if X-rays were electromagnetic waves then their wavelengths might be of the same order as the interatomic separation in crystals and diffraction would be observed. The original diffraction photograph was from a crystal of copper sulphate.

† Present address: ELETTRA, Sincrotrone Trieste, Trieste, Italy.

The essential feature of the Laue method, as it became called, is that the incident X-ray beam is polychromatic and the crystal sample is held stationary. All the X-rays emitted by an emission tube and passing through the tube exit window are allowed to impinge onto the sample; no special filtering or monochromatization is used. The Bremsstrahlung continuum and the characteristic emission lines constitute the incident spectrum of X-rays. This beam hits the stationary crystal and the spots making up the diffraction pattern arise from different wavelengths. A given reflecting plane in the crystal extracts from the beam the particular wavelength that allows constructive interference or reflection to occur. In contrast to the angular rocking width of a reflection in the monochromatic rotating crystal method, each reflection in the Laue method is stimulated by a small range of wavelengths whose mean wavelength lies somewhere in the broad range of incident wavelengths. The Laue method is described in the book by Amorós et al. (1975).

The first analyses of simple crystal structures were made using Laue photographs when W. L. Bragg deduced the structure of sodium chloride and other alkali halide crystals (for a historical review see Bragg (1975)). Subsequently, Bragg primarily used the monochromatic X-ray beam to illuminate the crystals in the spectrometer designed and built by his father W. H. Bragg. Keen proponents of the use of the Laue method were R. W. G. Wyckoff (1924) and L. Pauling. However, the Laue method at that time was subject to various limitations and its use lapsed as a method for determining molecular structure as it became superseded by the monochromatic rotating crystal method. In any case, the weakness, by four orders of magnitude, of the Bremsstrahlung radiation relative to the characteristic lines naturally meant that the use of the Laue method was limited to the study of well diffracting crystals.

The spectral curve of SR, however, ideally lends itself to Laue geometry since a broad band of wavelengths of high intensity is emitted by the synchrotron electron beam. As a result, a very large number of reflections can be recorded in a remarkably short exposure time in a Laue experiment; this has led to a revival of the Laue method as a means for quantitative structure analysis, especially for kinetic studies and the analysis of structural perturbations. Time resolved investigations include the study of enzyme crystals. For an application of the synchrotron Laue method to study GTP hydrolysis by the enzyme p21 see Schlichting et al. (1990). Other, more recent, examples of time-resolved macromolecular crystallography are described in Cruickshank et al. (1992). The fastest exposure time realized for Laue data recorded from a protein crystal is 120 psec at CHESS (Szebenyi et al. 1992), thus indicating the tremendous scope of these new methods for time-resolved studies. Facilities for Laue data collection also exist or are planned at SSRL (Stanford), NSLS (Brookhaven), PF (Tsukuba), EMBL (Hamburg), LURE (Paris), ESRF (Grenoble), APS (Argonne) and ELETTRA (Trieste).

In this paper a review is given first of the development of the Laue method and its use at the synchrotron showing how it has emerged as an important technique in modern crystallography. Its importance is perhaps not linked to a large number of cases but rather to a number sufficient to illustrate that studies of proteins in the crystalline state are of biologically reactive molecules. The paper then goes on to report a recent Laue crystal structure analysis, of a cobalt substituted concanavalin A, using image plate data recorded at Daresbury on station 9.5. Other experimental developments of the Laue method are then given. These involve the use of a large toast-rack and a mylar transmission mirror. A problem that remains with the Laue method, is that the low resolution data are incomplete. A new monochromatic

method known as LOT (large-angle oscillation technique), has been introduced by our group to alleviate this problem with a view to further improving protein crystal structure analyses with these rapid, synchrotron data collection, techniques. Reductions in exposure times both for Laue and LOT methods will come from harnessing the high brilliance insertion devices at ESRF, APS and SPRING-8 (Japan).

2. Historical objections to the Laue method

The Laue method was formerly considered generally unsuitable for intensity measurement and structure determination for several major reasons, which were as follows.

(*a*) The multiplicity or overlapping orders problem (W. L. Bragg 1975, p. 137; Wyckoff 1924, p. 142; Amorós *et al*. 1975, p. 13; Steinberger *et al*. 1977, p. 1260).

(*b*) The wavelength normalization problem (Bragg 1949).

(*c*) In the case of a protein crystal, the radiation sensitivity of the sample in the SR beam (Blundell & Johnson 1976; Greenhough & Helliwell 1983; J. C. Phillips, personal communication).

A discussion of these historical objections has been given by M. Helliwell (1989).

Additionally, in Laue geometry only cell ratios can be determined from the angular coordinates of spots. The absolute cell parameters therefore have to be provided from a monochromatic study.

We review in §§3, 4 and 5 the theoretical and experimental work, as well as Laue structure analyses, which have shown that these objections are not as serious as once thought.

3. Laue diffraction geometry

For a stationary crystal and white radiation with $\lambda_{max} \geqslant \lambda \geqslant \lambda_{min}$ the reciprocal lattice points (RLPs) whose reflections can be recorded lie between the Ewald spheres of radii $1/\lambda_{max}$ and $1/\lambda_{min}$. These spheres touch at the origin of the reciprocal lattice (figure 1*a*), and the wavelength at which any individual RLP diffracts is determined by the reciprocal radius of the Ewald sphere passing through it.

(*a*) *Energy overlaps*

A Laue diffraction spot can be composed of several RLPs and wavelengths such that for an RLP corresponding to a spacing d and a wavelength λ the RLPs with $\frac{1}{2}d$, $\frac{1}{3}d$, will also be stimulated by the respective wavelengths $\frac{1}{2}\lambda, \frac{1}{3}\lambda$, etc. That is, these orders of a Bragg reflection are exactly superimposed in angle. This was the problem referred to by W. L. Bragg and Wyckoff as mentioned above. Such a spot has a potential multiplicity determined by the resolution limit. However, the recorded multiplicity may be less. For example, figure 1*b* shows a ray with five orders inside the D^*_{max} sphere (the sample resolution limit), but of which only two are within the accessible region. The ray and corresponding Laue spot are therefore of actual multiplicity 2. However, contrary to the statements of W. L. Bragg and Wyckoff, the pattern is dominated by single wavelength, single RLP spots even if there is an infinite bandpass ($\lambda_{max} = \infty, \lambda_{min} = 0$).

If we consider a given reflecting plane of spacing d there is an associated set of Miller indices (h, k, l). Now $(2h, 2k, 2l)$ may be beyond the resolution limit and (h, k, l) may also have no common integer divisor. Hence, the Laue spot that results will contain (h, k, l) only.
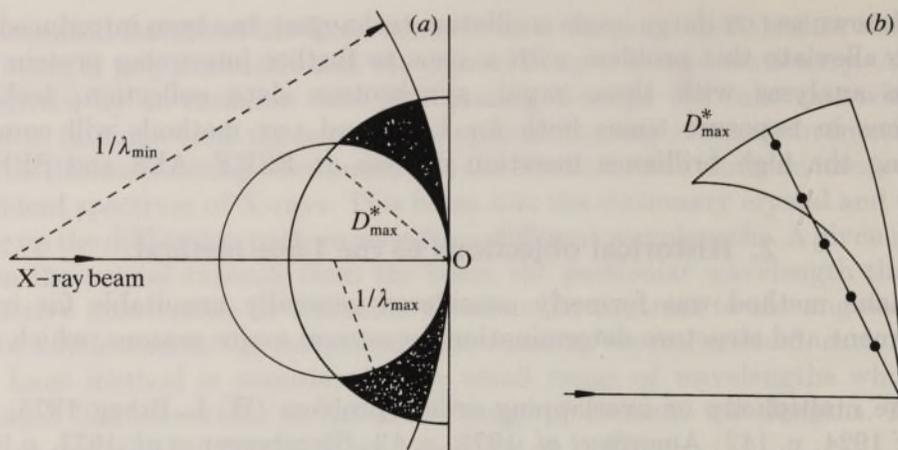
Figure 1. (a) Laue diffraction geometry showing the accessible region of reciprocal space between the Ewald spheres associated with λ_{min} and λ_{max} and the sample resolution limit D^*_{max}; O is the origin of reciprocal space. (b) A ray with n orders inside the D^*_{max} sphere can have a recorded multiplicity $m < n$ where $(n-m)$ RLPs are outside the accessible region. The diagram shows the case of $n = 5$ and $m = 2$. Only the upper section of the volume of resolution of the accessible region is shown. From Cruickshank *et al.* (1987).

It can be shown (Cruickshank *et al.* 1987) that the probability that a randomly chosen RLP has no common integer divisor is

$$Q = \left(1 - \frac{1}{2^3}\right)\left(1 - \frac{1}{3^3}\right)\left(1 - \frac{1}{5^3}\right)\left(1 - \frac{1}{7^3}\right)\left(1 - \frac{1}{11^3}\right)\cdots$$

$$= 0.83191\ldots. \tag{1}$$

To explain this expression one needs to understand the properties of integers, especially primes. The probability that an integer, h, is divisible by another integer, p, to produce an integer is $1/p$. The probability that three integers h, k and l are each divisable by the same integer, p, is $1/p^3$ and the probability that (h, k, l) do not have a common integer divisor, p, is $1 - 1/p^3$. To exclude the possibility of any value of p being a divisor the product series giving $Q = 0.832$ involves the infinite set of terms based upon prime numbers only. In the case of $\lambda_{max} = \infty$ and $\lambda_{min} = 0$ all RLPs lying between $\frac{1}{2}D^*_{max}$ and the origin of reciprocal space will be recorded as part of a multiple Laue spot. For those RLPs lying between D^*_{max} and $\frac{1}{2}D^*_{max}$ there is a probability Q that they will be recorded as singles; this region is $\frac{7}{8}$ of the resolution sphere volume. Hence, a proportion of all RLPs $= \frac{7}{8}Q = 72.8\%$ lie on single rays and a proportion $\frac{7}{8} = 87.5\%$ of all rays (Laue reflections) are single rays. Similarly, $2(1/2^3 - 1/3^3)$ $Q = 14.6\%$ of all RLPs lie on double rays, whereas $(1/2^3 - 1/3^3) = 8.8\%$ of all rays are double rays. In the case of a more restricted bandwidth, the experimental situation, the proportion of singles increases.

A probability map in reciprocal space can also be constructed for the general case (finite λ_{max} and λ_{min}) giving details of the likelihood of RLPs being recorded as single, double, triple, etc. (see figure 2a, b). A striking feature of the probability map for singles, for example, is the region of zero probability between $\frac{1}{2}D^*_{max}$, and the origin, on the one hand and $2\lambda_{min}$ and λ_{max}, on the other.

Moreover, since the number of RLPs sampled is given by

$$\pi D^*_{max}(\lambda_{max} - \lambda_{min})/4V^* \quad \text{for} \quad \lambda_{max} < 2/D^*_{max}, \tag{2}$$
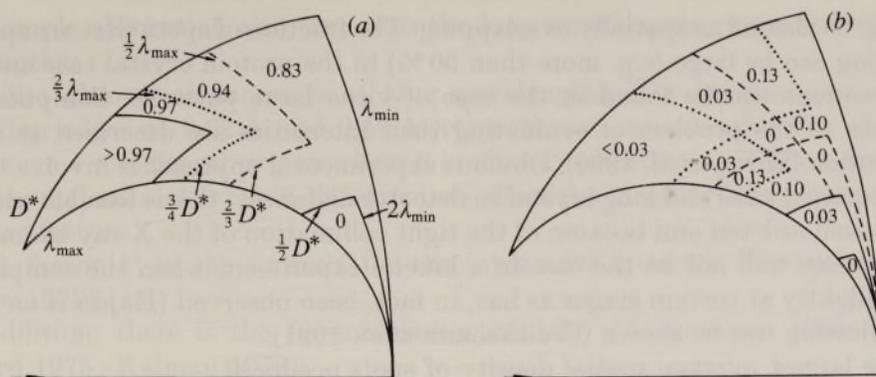
Figure 2. The probability of an RLP being recorded in (a) a singlet Laue spot and (b) a doublet Laue spot in different regions of reciprocal space. The wavelength labels indicate which Ewald sphere is appropriate. The actual radius of each Ewald sphere is the reciprocal of the label shown. The labels for the resolution spheres, e.g. D^*_{max} are true distances on the diagram. From Cruickshank *et al.* (1987).

where V^* is the reciprocal unit cell, the variation of D^* to the fourth power shows that the volume of low resolution data sampled is actually quite small. The number of RLPs within the resolution sphere is

$$\tfrac{4}{3}\pi D^{*3}_{max}/V^*. \tag{3}$$

The Laue method therefore appears very effective at sampling data between D^*_{max} and $\tfrac{1}{2}D^*_{max}$. These RLPs are largely recorded as singles. The effective sampling of the region between $\tfrac{1}{2}D^*_{max}$ and the origin of reciprocal space needs a method to achieve the separation of the component reflections in a spot. There are several possible methods available to achieve this. The process is often referred to as wavelength or energy deconvolution. These methods include the following.

(a) The use of multiple films to record a Laue pattern. The variation of the film adsorption factor with wavelength means that the different components of (and wavelengths in) a Laue spot are attenuated at different rates (Zurek *et al.* 1985; Helliwell *et al.* 1989a, b).

(b) In the case of high symmetry it is possible that an RLP recorded in one place as a component of a 'double' spot may also occur elsewhere as a 'single' spot. In this case, the other component of a double may therefore be derived. Alternatively, in the case of multiples an RLP may occur several times, stimulated by different wavelengths. From these, it may be possible to extract the intensities uniquely associated with each RLP. Obviously, if there are a maximum of n RLPs in a spot there would need to be at least n unique observations.

(c) Recently the use of the Patterson function and direct methods has been made to estimate the components of doublet spots using experimentally recorded singles (Hao *et al.* 1993). Likewise, electron density map modification techniques could be quite powerful actually both for energy deconvolution and retrieving missing (i.e. unrecorded) reflections. For a recent discussion of such techniques applied to MIR (multiple isomorphous replacement) maps, see Cowtan & Main (1993).

(b) *Spatial overlaps*

The Laue diffraction spots on a film are of a certain size dependent on factors such as collimator size; crystal mosaic spread and beam divergence (Andrews *et al.* 1987; Helliwell *et al.* 1989a). If a spot encroaches on its nearest neighbour then these Laue

spots may be classed as spatially overlapping. The fraction of spots that are spatially overlapping can be large (e.g. more than 50%) in the protein crystal case and even higher fractions can be found in the case of virus Laue patterns. Computational approaches to the problem of evaluating their intensities are described as spatial deconvolution (Shrive *et al.* 1990). Obvious experimental approaches involve the use of large detector sizes and long crystal to detector distances; this is feasible when the sample is well ordered and because of the tight collimation of the X-ray beam. This, of course, may well not be the case in a kinetic experiment when the sample may disorder slightly at certain stages as has, in fact, been observed (Hajdu *et al.* 1987).

The following can be shown (Cruickshank *et al.* 1991).

(*a*) The largest average spatial density of spots occurs at

$$\theta_c = \arcsin \left[\lambda_{\min}/2d_{\min}\right]. \tag{4}$$

(*b*) The largest local density of spots occurs along the arcs approaching nodal spots. (Nodal spots are of low h, k, l values or multiples thereof and are associated with principal zones of the lattice.)

(*c*) The nearest neighbours to a nodal spot are single (see also Jeffery 1958).

(*d*) Most overlaps occur between singles. This explains why the population of spatial overlaps is essentially distinct from the population of energy overlaps.

The spatial overlap problem is not as fundamental an obstacle as the energy overlap problem once appeared to be. In small molecule Laue patterns there are virtually no spatial overlaps. However, in macromolecular crystallography it is a matter for serious concern (Cruickshank *et al.* 1991).

There is an optimum distance from the crystal for a standard film cassette. As the film is moved further back the number of spots which are no longer spatially overlapped is balanced by those spots that are lost as they pass beyond the edges of the film.

An experimental method to solve a significant part of the spatial overlap problem has been proposed (Helliwell 1991). By arranging films in a special cassette with a pre-set gap between them (e.g. a few centimetres) the front film allows quantitation of the long λ, high θ spots and the short λ spots can penetrate and expand to fill the whole aperture on the back film(s). Such an arrangement is referred to as a toast-rack (see §7).

4. Analysis of Laue data and wavelength normalization

The wavelength normalization problem was introduced in §2. The measured intensity of a single Laue spot or of an energy deconvoluted spot needs correction or normalization for a variety of wavelength-dependent factors if data are to be obtained comparable to monochromatic data. The wavelength at which a reflection is stimulated affects the measured spot intensity through a variety of processes.

(*a*) The intensity spectrum which is incident at the sample. The calculated spectrum is modified at the long wavelength end by absorption due to beam line windows or any airpaths in the Laue camera. Also the short wavelengths are cut off if a reflecting mirror is used (and this may be the case for focusing or to aid more precise definition of spot multiplicity).

(*b*) The source polarization state varies with wavelength (see Papiz & Helliwell (1984) for the SRS example).

(c) Sample absorption also truncates the long wavelengths compared with short wavelengths.

(d) Film absorption increases at long wavelengths. The bromine and silver K absorption edges at 0.92 Å and 0.49 Å, respectively, enhance the film absorption efficiency in a wavelength region where it is otherwise decreasing (Clifton *et al.* 1985).

(e) Anomalous scatterers in the structure affect the magnitude (and phase) of the structure factors.

(f) A factor λ^4 in the formula for the integrated intensity (Zachariasen 1945; Kalman 1979).

In addition, there is the Lorentz factor $(\sin^2\theta)^{-1}$ (Zachariasen 1945; Buras & Gerward 1975; Kalman 1979).

The recorded Laue intensity (strictly, integrated power) is given by Campbell *et al.* (1986) and references cited therein, for the reflection *hkl* (abbreviated to the symbol *h*,

$$I_L(\boldsymbol{h}) = \frac{e^4}{m^2 c^4} I_0(\lambda)\,\lambda^4 \frac{1}{2\sin^2\theta}\frac{V_x}{V_0^2} PAD\,|F(\boldsymbol{h})|^2. \tag{5}$$

Here, $I_0(\lambda)$ denotes the spectral intensity distribution of the incident X-ray beam; V_x is the volume of sample illuminated; V_0 is the sample unit cell volume; θ is the Bragg angle for the reflection $\boldsymbol{h}$; P is the polarization factor; A is an absorption correction for the sample in its capillary and D is a detector sensitivity and obliquity factor. Quantities such as P, A and D vary with any or all of λ, θ and x, the position of the diffracted beam on the detector; the spectral intensity distribution is, in general, not precisely known in advance; and the detector may suffer from spatial distortion and non-uniformity. Thus equation (5) may be written as

$$I_L(\boldsymbol{h}) = KG(\lambda, \theta, x)|F(\boldsymbol{h})|^2, \tag{6}$$

where K is a constant. Assuming that all quantities which depend on more than one variable are factorable then $G(\lambda, \theta, x) = f(\lambda)\,g(\theta)\,j(x)$, and

$$|F(\boldsymbol{h})|^2 = [Kg(\theta)j(x)]^{-1}[f(\lambda)]^{-1}I_L(\boldsymbol{h}). \tag{7}$$

It is reasonable to assume that K, $g(\theta)$ and $j(x)$ are known, to a very good approximation. Thus, the quantifying of Laue diffraction patterns depends almost entirely on determination of $f(\lambda)$, known as the wavelength normalization curve or λ-curve.

The methods that have been used to derive a wavelength normalization $f(\lambda)$, curve are as follows.

(i) The use of a silicon standard crystal and a film in a pilot study on aluminium phosphate (Wood *et al.* 1983).

(ii) The use of monochromatic data as a reference set for the same sample (Helliwell *et al.* 1989a).

(iii) The use of symmetry equivalent reflection intensities measured at separate wavelengths (Campbell *et al.* 1986; Helliwell *et al.* 1989a). D. W. J. Cruickshank (1992) has pointed out that a similar method was used by Ewald (1914). Similar information is also available when the same reflections are measured on several film packs with different crystal orientations.

Methods (ii) and (iii) have generally been the ones used to process protein and small molecule crystal Laue data. The protein crystal processing statistics are almost as good as monochromatic oscillation camera data (Machin & Harding 1985; Helliwell

et al. 1986, 1989 *a*; Machin 1987; Temple & Moffat 1987). Method (iii) breaks down in the presence of strong anomalous scattering; in such a case either method (ii) can be used or a λ-curve derived by method (iii) from a non-anomalously scattering crystal structure; alternatively, in trying to derive a data set that is completely equivalent to a monochromatic one with many measurements of a reflection at different wavelengths it should be possible, in principle, to derive the wavelength independent and wavelength dependent parts of the structure factor separately using the method of Karle (1980).

In the case of monitoring a sample of known structure via the Laue pattern it is possible to use the fractional differences whereby the wavelength-dependent corrections cancel. This difference ratio method can be used with time-resolved or perturbation measurements. A simplification, in principle, of the time-resolved Laue experiment over its static counterpart now appears: only fractional changes in intensities are needed (Bilderback *et al.* 1984; Hajdu *et al.* 1987).

5. Results establishing the credentials of the method

SR Laue data were recorded from a crystal of aluminium phosphate and analysed by Wood *et al.* (1983). The *R*-factor on *I* in the Laue data refinement was 19% compared with a conventional monochromatic single crystal study (Thong & Schwarzenbach 1979) with an *R*-factor of 2.2% of *F*. For the Laue data 20% of the measurements were rejected on the basis of bad intensity agreements. Of the eight refined positional parameters six were within 2σ and only one outside 3σ. The temperature factors were somewhat less well determined. This was considered to be due to the significant extinction and multiple diffraction effects for a hard material such as aluminium phosphate.

Laue data from a protein crystal were first recorded by Moffat *et al.* (1984, 1986) who used a somewhat restricted wavelength range to avoid the multiplicity problem. It was established that rapid exposure times were feasible.

Broad bandpass Laue data were recorded from a crystal of the protein pea lectin using the SRS wiggler (Hails *et al.* 1984; Helliwell 1984, 1985). These data were used in a pilot study to assess protein crystal Laue data quality. This protein crystal is relatively radiation insensitive. Overall merging statistics for the Laue data after wavelength normalization (Campbell *et al.* 1986) were almost as good as monochromatic data. The merging *R*-factors on intensity for Laue and monochromatic pea lectin data were respectively 8.2% and 6.3%. The mean fractional difference on *F* of the Laue data scaled to monochromatic data was 12.9% to 2.6 Å resolution. These results are summarized in Helliwell *et al.* (1989 *a*). The best quality data occur in the λ range 0.59 Å $< \lambda <$ 1.57 Å (Helliwell *et al.* 1989 *b*). At the short wavelength end the photographic films become increasingly transparent and at the long wavelength end sample absorption increases. Both effects weaken the diffraction spot. Sample absorption also introduces systematic errors at long wavelengths.

A difference Fourier map was calculated for the enzyme glycogen phosphorylase *b* using synchrotron Laue data (Hajdu *et al.* 1987, 1988). The map showed electron density for maltoheptose at the binding site.

The sensitivity of the Laue method to find weak scattering centres such as hydrogen in a small molecule crystal structure has been shown (M. Helliwell *et al.* 1989).
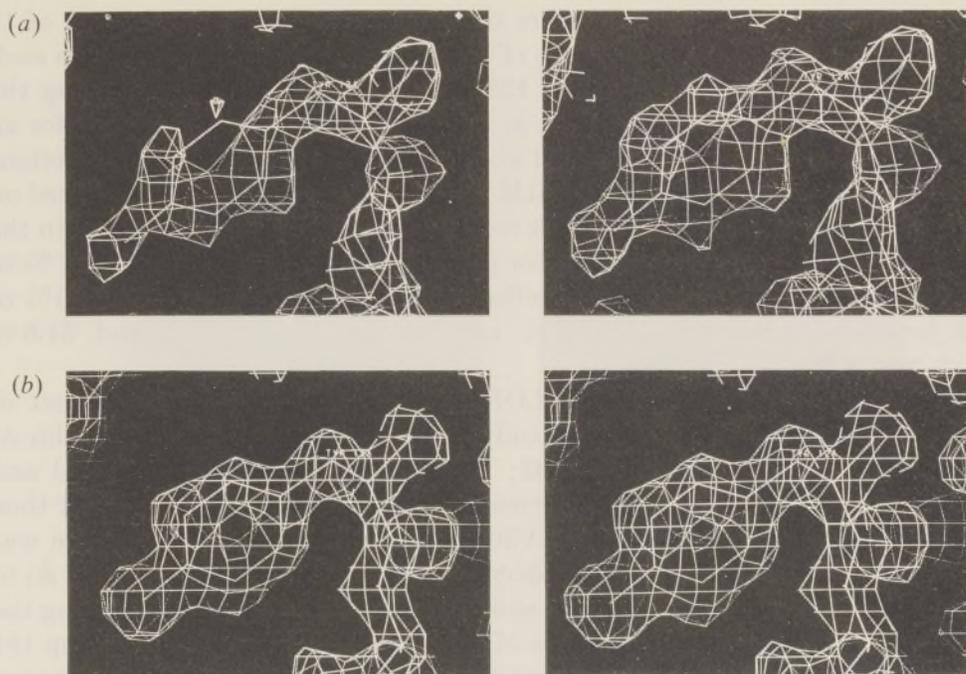
(a)

(b)

Figure 3. (2Fo − Fc) electron density maps for cobalt substituted concanavalin A. (a) Laue image plate data (d_{min}, 2 Å) contoured at 1.5σ (left) and 1.0σ (right) (b) monochromatic image plate data (d_{min}, 1.6 Å) contoured at 1.5σ (left) and 1.0σ (right). The Laue and monochromatic maps agree in the essential features. Continuity of the electron density in the Laue map is somewhat affected by missing low resolution data.

A study on a low pH form of carbonic anhydrase (Lindahl *et al.* 1992) has shown that a sensitivity to the scattering of one water molecule in 29000 Da of enzyme is possible with the synchrotron Laue method.

A time-resolved synchrotron Laue experiment has been performed by Schlichting *et al.* (1990) to study GTP hydrolysis in the ras p21 protein.

A survey of the methods of time-resolved macromolecular crystallography and structural examples are given in Cruickshank *et al.* (1992).

6. Refinement of Co concanavalin A using Laue data recorded with an image plate

The previous studies described in §5 all used photographic film as detecting medium. The performance of film can be improved upon by use of an image plate in terms of dynamic range, lower noise and better absorption efficiency. We have performed a study of a Co substituted concanavalin A crystal (space group I222, cell parameters, $a = 88.7$, $b = 86.5$, $c = 62.5$ Å) using Laue data recorded on a MAR Research (Hamburg) image plate system on station 9.5 at Daresbury (Brammer *et al.* 1988). A relatively narrow bandpass centred at a short wavelength (0.5 Å $< \lambda < 0.9$ Å) was used so as to obtain data to high resolution (2 Å). In a separate study (Weisgerber & Helliwell 1993) we have found that such a wavelength band, centred at short wavelength, reduces the background noise in the pattern and so enhances the resolution limit of the data from 2.4 Å to 2.0 Å compared with use of a broad bandpass (0.5 Å $< \lambda < 2.6$ Å).

With the SRS operating at 2 GeV and 230 mA, a crystal of thickness 0.5 mm, and

a collimator of 0.2 mm diameter, exposure times were typically 10 s. A total of 15 exposures were recorded each at an angle of between 7° or 10° with respect to each other, covering a total angular range of 122°. The data were processed using the Daresbury Laue software suite (described in Helliwell *et al.* (1989*a*)) modified for an image plate (J. W. Campbell, unpublished work). A total of 39453 singlet reflections were accepted by the program LAUENORM for wavelength normalization based on the use of symmetry equivalent reflections recorded at different wavelengths. In the program AGROVATA the merging R factor (on intensity) for the data was 7.7% to 2.0 Å. The total number of independent reflections was 12723 comprising 76.5% of the total between $\infty > d > d_{min}$, 80.5% between $2d_{min} > d > d_{min}$ and 51.6% between $\infty > d > 2d_{min}$.

The structure was refined using X-PLOR (version 2.1) with a starting set of coordinates from a monochromatic 2 Å study of a Cd substituted concanavalin A, also in space group I222 (Naismith 1992; Naismith *et al.* 1993). Positional and temperature factor refinement was performed. Map calculation followed and then model building using FRODO on an ESV30 graphics system. The procedure was repeated until convergence. The R factor decreased from an initial 20.9% (8–2 Å) to 17.1%, with rms-bond lengths = 0.012 Å and rms-bond angles = 2.984°. During the model building, the gene sequence changes of Min *et al.* (1992) at Glu 151 to Asp 151 and Arg 155 to Glu 155 were clearly revealed. Some waters were deleted and new ones were inserted. There was no evidence of a second cobalt site as suggested in the cadmium structure. The cobalt and calcium atoms were 4.15 Å apart and had environments comparable to that of a monochromatic structure study conducted at a resolution of 1.6 Å. Figure 3 compares (2Fo-Fc) maps at an aromatic residue (Tyr 12) of the structure based on (*a*) the Laue image plate data (2 Å resolution) and (*b*) monochromatic image plate data (1.6 Å resolution) showing that the Laue map is certainly of readily interpretable quality.

7. Recording of Laue data using a large toast-rack film arrangement

An earlier paper has reported the measurement of Laue data on a toast-rack constructed for films of size *ca.* 118 mm × 118 mm (Helliwell 1992). These data on native concanavalin A have been used to refine the structure at 2 Å resolution (Weisgerber & Helliwell 1993). The purpose of the toast-rack is primarily to alleviate the problem of spatial overlaps in Laue diffraction of macromolecular crystals. An extra benefit is that the rear films have a very low background.

Since standard microdensitometers accept larger films and since there is a larger film size (240 mm × 180 mm) conveniently available from commercial suppliers, a large toast-rack has been constructed (see Helliwell 1992, fig. 3). This has now been used successfully to record Laue diffraction data from a native concanavalin A crystal. Figure 4 shows four films placed at distances of 104, 164, 304 and 504 mm from the crystal respectively. Clearly, this new toast-rack looks especially effective at dealing with the spatial overlap problem. Further modifications of the Daresbury Laue Software are now needed to process these larger films. Results will be reported at a later date.
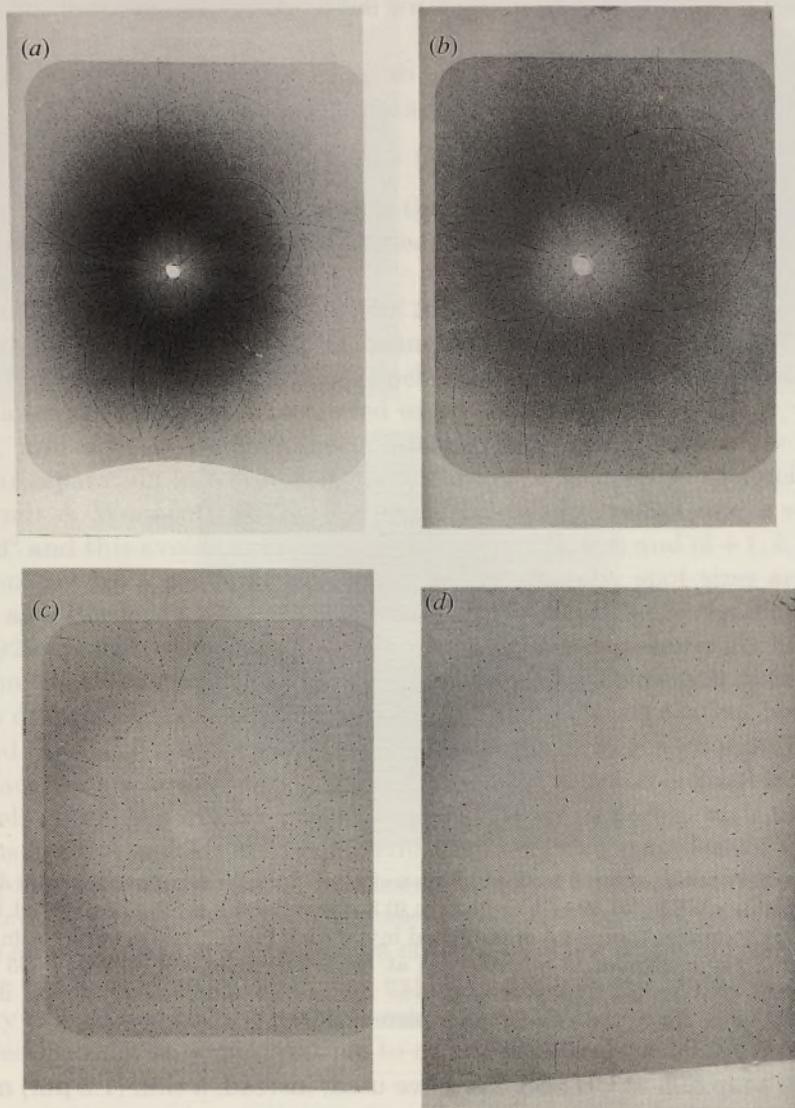
Figure 4. Four Laue diffraction films (each of size 240 × 180 mm), exposed in a large toast-rack, set respectively at distances of (a) 104, (b) 164, (c) 304, (d) 504 mm from a crystal of the protein concanavalin A on station 9.5 of the Daresbury SRS (operating at 2 GeV, 200 mA, 0.2 Al foil in the beam). CEA Reflex 15 photographic film.

8. Development of a transmission X-ray mirror for control of λ_{max} in the Laue method

The use of a restricted wavelength range, centred at short wavelength ($0.5 < \lambda < 0.9$ Å), has been found to be useful for high resolution data collection from concanavalin A (Weisgerber & Helliwell 1993). The λ_{max} cut off was established using a 1 mm thick Al filter placed in the beam. This unfortunately also leads to attenuation of the beam intensity in the wavelength range of interest and significantly increases the required exposure time.

An alternative method is to use a transmission X-ray mirror. Lairson & Bilderback
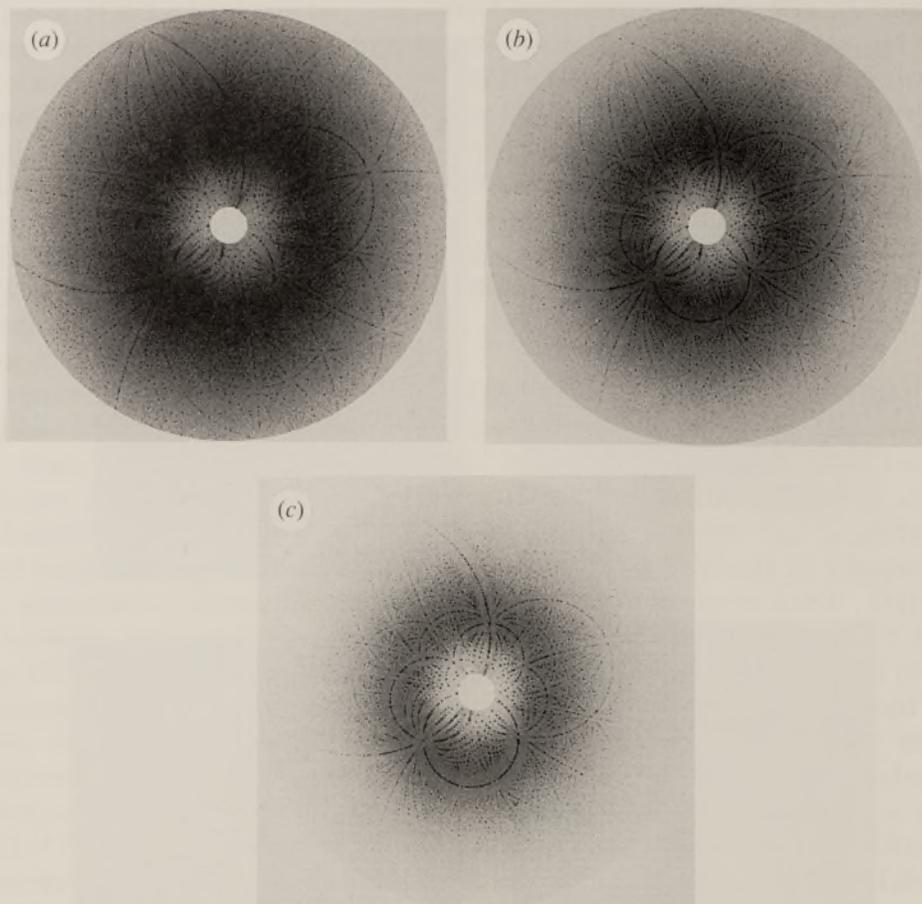
Figure 5. Laue diffraction patterns recorded from a crystal of the protein concanavalin A on station 9.5 of the Daresbury SRS. (*a*) Broad band-pass ($0.5 < \lambda < 2.6$ Å), (*b*) λ_{max} restricted by use of a transmission X-ray mirror composed of stretched mylar film, (*c*) λ_{max} restricted by using a 0.2 mm Al foil instead of the transmission mirror. SRS at Daresbury operated at 2 GeV, 35 mA (single bunch). For each pattern the exposure time was 100 milliseconds, the crystal to image plate distance was 150 mm, the radius of the image plate was 90 mm and $\lambda_{min} = 0.5$ Å.

(1982) used a soap film at CHESS. We have used, instead, a thin (1.5 µm) mylar film stretched over a wire frame in tests at Daresbury which are reported here. Figure 5 compares three Laue patterns recorded from the same concanavalin A crystal, in an identical orientation, both without and with such a transmission mirror in place and also with a 0.2 mm Al foil instead of the transmission mirror. The effect of the reduction of λ_{max} with the transmission mirror and the Al foil is evident in the high angle parts of the pattern. Data processing is in progress and these results will be reported elsewhere.

9. Development of a new, monochromatic, large-angle oscillation technique

In §3*a* the problems associated with the Laue method in sampling the low resolution data were referred to. This problem was discussed at the Royal Society Discussion Meeting held in January 1992 on the topic of time-resolved macromolecular crystallography. David Blow remarked that 'the low resolution data,

being the strongest and least affected by radiation damage, should be the easiest to measure'. So, how should this be done with minimal effect on the time needed to measure data?

Helliwell (1992) provided an analysis of the time-resolved aspects of data collection, e.g. the total time, T, to measure a data set is given by

$$T = [Nt_1 + (N-1)t_2], \tag{8}$$

where N is the number of exposures, t_1 is the time for each exposure and t_2 the time needed to change a film or toast-rack or clear/refresh a detector image. Clearly T can be reduced if $N = 1$!

Hence, a single exposure is very helpful for rapid data collection. To avoid energy overlapped spots a monochromatic beam is required. Can these two needs be combined? Traditionally, in the monochromatic rotation method the angular rotation range per exposure is restricted to a value, $\Delta\phi_{max}(\approx a^*/D^*_{max})$, where D^*_{max} is $1/d_{min}$, and d_{min} is the minimum interplanar spacing, and $a^*(= 1/a)$ is the interplanar separation in reciprocal space when a^* is parallel to the incident X-ray beam (Arndt & Wonacott 1977). For example, for $d_{min} = 3$ Å and $a = 60$ Å then $\Delta\phi_{max} \approx 3°$ and this avoids overlap of the two spots (h, k, l) and $(h+1, k, l)$. This is a requirement set by a conventional X-ray source whereby spot sizes are relatively large (see also Bernal 1927, p. 283) and the background noise is significant (see also Bernal 1929, p. 349) even on a 3° exposure. At the synchrotron, however, the collimation, short wavelength and intensity combine to yield small diffraction spots and clean diffraction patterns. In particular, the angular separation between spots (h, k, l) and $(h+l, k, l)$ is sufficiently large to allow much larger rotation ranges to be used. In fact, for low-resolution data collection, a 90° rotation is feasible. Weisgerber & Helliwell (1993) report the first examples of such patterns, with tests done to resolutions of 4.8 Å and 3.9 Å. The patterns were very clean, not least due to the long crystal-to-film distances required for these resolution limits. Even for the case of a well set crystal a large fraction of the spots were resolved one from the other but there was an advantage in misaligning a crystal, by a few degrees, from a particular setting or use a random setting. The exposure time was 27 min per exposure at Daresbury which is quite long for time-resolved work. However, prospects at ESRF exist to reduce such an exposure time to a few seconds for a 90° sweep.

10. ESRF

The ESRF is a high energy (6 Gev) high brilliance synchrotron radiation source with a large circumference allowing for the insertion of multipole wiggler and undulator devices emitting in the X-ray range. The machine has recently been commissioned successfully. Beamline commissioning has now commenced (Witte 1993; Riekel, Kvick & Grübel, personal communication). A beamline for Laue diffraction at ESRF is described by Wulff (1991).

11. Concluding remarks

W. L. Bragg (1975) in the frontispiece of his book *The development of X-ray analysis* compared a model of sodium chloride with that of the protein haemoglobin to illustrate the power of X-ray analysis in tackling complex as well as simple structures. This complexity has now extended to much larger molecular assemblies

such as viruses. This latter development is, in fact, one of the major contributions of SR. Rapid SR data collection techniques have also opened up the field of time-resolved macromolecular crystallography, thus enabling the study of reactivity as well as structure. The problems and challenges faced by W. L. Bragg and the other early pioneers of X-ray diffraction have, in a way, been revived with the advent of synchrotron radiation. However, the rewards for using the intense, polychromatic SR beam are far greater than those for using the weak continuum background from a conventional X-ray tube. Moreover, computers are now available to assist the modern crystallographer thus allowing relatively easy data processing, simulation and retrieval. Hence, these advantages and the use of the synchrotron have led to the emergence of the Laue method and other new techniques for X-ray analysis thus extending considerably its scope.

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