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Time-Resolved Macromolecular Crystallography: Introductory Remarks and a Little History

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Time-resolved macromolecular crystallography: introductory remarks and a little history†

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X-ray crystallographic data can be collected rapidly at synchrotrons by the Laue method. The early use of the Laue method by Ewald, Nishikawa, Wyckoff and Pauling is reviewed.

1. Introductory remarks

X-ray diffraction studies have made outstanding contributions to structural molecular biology. The advent of high-intensity synchrotron radiation sources, coupled with the revival of the Laue method, has now made possible the rapid collection of X-ray crystallographic data. In the Laue method, roughly speaking, the full polychromatic spectrum of the source is unleashed at a stationary crystal. There is no throwing away of most of the X-ray photons to achieve a quasi-monochromatic incident beam, nor is there any rotation of the crystal.

Laue data-sets have been collected in acquisition times ranging from seconds to milliseconds. In special demonstrations single-bunch exposures of 120 ps have yielded valid data-sets.

Thanks to synchrotron radiation, protein and virus crystallography is now progressing from studies of equilibrium structures to time-resolved studies of structures at reaction stages. Many enzymes are catalytically active in the crystal. The first results on the structures of enzyme intermediates have been reported. If the half-life of a particular crystalline state is a few minutes or more, data can be gathered with monochromatized synchrotron radiation by rotating crystal methods. For states with lifetimes of less than a minute, the Laue method is essential.

The full exploitation of the synchrotron Laue method for the study of dynamic events in crystals requires many developments in physics, chemistry and biochemistry.

What important biochemical processes can be studied in the crystal? How can reactions be initiated uniformly and simultaneously throughout the crystal? How can reactions be monitored? How can the timescales of X-ray data acquisition be adjusted to the timescales of biochemical processes, and vice versa? How can mixed-conformation structures be analysed? How can X-ray techniques be advanced? What will be possible with the next generation of synchrotrons, now little more than a year away?

The papers presented in this volume aim to report some of the striking results that have already been obtained and to discuss and identify necessary future developments.

† Dedicated to Ralph W. G. Wyckoff and Linus Pauling, Foreign Members of the Royal Society, pioneers of the Laue method.

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2. A little history of the Laue method

The Laue method – white radiation, stationary crystal – was the method used by Laue and his colleagues in 1912 in the original discovery of X-ray diffraction. It was used by W. L. Bragg in his earliest structure determinations, but W. H. Bragg and W. L. Bragg came quickly to use the ionization spectrometer with monochromatic radiation, and with the crystal and ionization chamber mounted on coaxial turntables. Meanwhile in Munich important developments of the Laue method were made by Ewald (1914*a*, *b*). He introduced the concept of the parameter-dependent *structure factor*, and in the structure analysis of pyrite FeS_2 the coordinate parameter was varied so as to match the observed intensity ratios for pairs of planes of the same spacing. In the second paper, he showed how *unsymmetrical* photographs of zincblende ZnS could reveal the wavelength dependence of intensities. With an unsymmetrical crystal orientation, planes of the same form with symmetrically equivalent indices reflect at different wavelengths. Thus a comparison of their intensities shows the spectral curve.

These two techniques of Ewald were picked up and developed across the world; in Japan by Nishikawa (1915). He passed them on, plus space group theory, with 'untiring advice and aid' to Wyckoff (1920*a*) at Cornell during a visit to the U.S.A. in 1917–18. A little later Wyckoff helped Dickinson, who in turn taught Pauling when he arrived in Pasadena as a graduate student in the autumn of 1922. Wyckoff and Pauling were responsible for the very effective use of the Laue method in structure analysis in the U.S.A. in the 1920s.

Remarkably, Pauling and Wyckoff are now the two most senior Foreign Members of the Royal Society. Pauling was elected in 1948, and Wyckoff was elected in 1951. Appositely, not only were they pioneers of the Laue method of structure analysis, but both have made immensely important contributions to studies of biological macromolecules. In the latter part of 1991 I corresponded with them both. They sent their best wishes to the participants in the Discussion Meeting, and these greetings provided a felicitous opening to the proceedings.

Pauling further wrote:

> 'I have followed the development of diffraction methods in crystallography for 70 years, beginning two or three years after Ralph Wyckoff [Wyckoff (1920*a*)] had carried out his first structure determination, on cesium dichloroiodide. Wyckoff, Nishikawa, and Ewald had developed the method of using Laue photographic data. I was taught this method, including the use of rigorous, rational arguments, by Roscoe Gilkey Dickinson in 1922, when I arrived in the California Institute of Technology to begin my work as a graduate student. Wyckoff had been there throughout the preceding year. At that time the procedure based on analyzing Laue photographs was by far the most reliable method in X-ray crystallography. During the following decade I published many X-ray structure determinations made with the use of this procedure, and also about a dozen papers in which I corrected structures reported by other X-ray crystallographers.'

For hexamethylenetetramine, in the very first X-ray structure determination of an organic compound, Dickinson & Raymond (1923) determined the carbon and nitrogen positions within 0.02 Å of the modern values (Becka & Cruickshank 1963).

One of the tools that is now proving very helpful in the interpretation of

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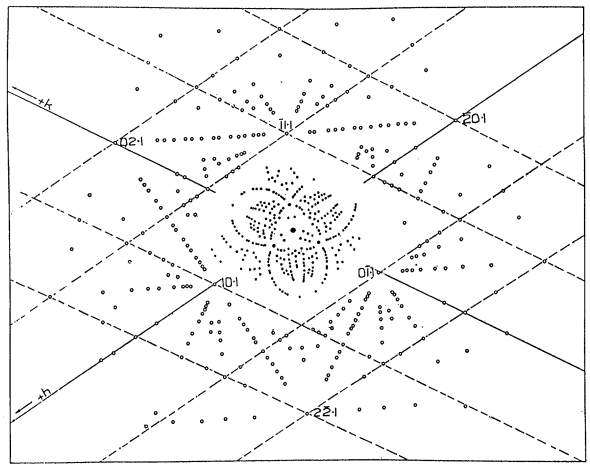

Figure 1. Wyckoff's (1920*b*) gnomonic projection of the Laue pattern obtained by passing X-rays through a basal (111) section of rhodochrosite $MnCO_3$. The Laue pattern is at the centre of the figure, and the gnomonic projection was constructed with the aid of a ruler as described in Pauling's letter. The spots on the Laue pattern correspond to angular coordinates $(2\theta, \beta)$, where θ and β are the Bragg and azimuthal angles. The gnomonic projection transforms the spots to $(\pi/2-\theta, \pi+\beta)$ and projects them on a plane normal to the incident beam. The gnomonic projection provides an undistorted wide angle view of the reciprocal lattice as seen from its origin; as a result the zone conics of the Laue pattern become straight lines in the projection.

synchrotron Laue patterns is the gnomonic projection (Cruickshank *et al.* 1992; Carr *et al.* 1992). Wyckoff wrote:

> 'It is very pleasing to find that the Gnomonic Projection which more than 70 years ago was a life saver in my dealing with Laue photographs is still in good use. Crystallography has so drastically changed with time that I find myself surprised by reference to anything that was for us a vital problem. Your noting my 1924 book in this connection led me to see if I still had a copy of the journal article that described my first use of the gnomonic.'

He then enclosed a copy of his wide-ranging paper (Wyckoff 1920*b*), from which the gnomonic projection in figure 1 is taken.

Interestingly, there is no reference either in this paper or in the text of his book (Wyckoff 1924) to the reciprocal lattice (Ewald 1913, 1921), of which the gnomonic projection is an immediate representation (Ewald 1923). Wyckoff (1920*b*) simply said: 'The positions of the *normals* to the *planes* which are reflecting the X-rays are plotted in the gnomonic projection', and gave references to classical papers.

Later in 1991 Wyckoff wrote to me:

> 'It has certainly been an unanticipated pleasure to see how the introduction of synchrotron radiation has made Laue photographs again the source of much valuable information. I am also very glad this is drawing attention to Dr Nishikawa's major contributions to their interpretation.
>
> I am not sure when I first became aware of the reciprocal lattice but it certainly was sometime after I had finished writing my *Crystal structures*. Scientific relations with Europe were fragmented not only during the course of the First War but for a long time thereafter. W. L. Bragg's 1913 paper was not available in Ithaca till 1916 and I made contacts with German science only after

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being in the Geophysical Laboratory. They began with Professor Schoenflies as the developer of space group theory. Relations with Ewald grew rapidly once we met and continued till his recent death but they never involved questions dealing with dynamic theory.'

In a second letter Pauling gave more details of the methods he had used:

'In the fall of 1922 Dickinson showed me that I should prepared unsymmetrical Laue photographs, tipping the crystal a little away from the symmetry axis. We made gnomonic projections on heavy paper, each projection on a sheet about three feet square. During the first year or two I used a ruler that was graduated in centimeters. This ruler was replaced by another one, invented by Maurice Huggins, in which the rulings gave the values of $\sin\theta$. This small change saved one step in the interpretation.

I may have made more than one hundred of these gnomonic projections, and my students made perhaps a hundred or two. In my early research books, now in the Ava Helen and Linus Pauling Archives, Oregon State University, Corvallis, Oregon 97331-6202, there are perhaps a thousand pages on which there are listed the values of $\sin\theta$, estimated intensity and values of $n\lambda$. Sometimes I plotted the intensities of the planes of a form against $n\lambda$ and drew a curve through them. Neither Dickinson nor I interpolated intensities very often. Instead, we looked for pairs of spots from different forms with nearly the same value of $n\lambda$, in range usually from 0.24 to 0.48 Å (first order)'. (This allowed the observed intensity relation for a pair to be compared with the calculated intensity relation without the complication of wave length dependent factors.)

The usefulness of the wave length range 0.24–0.48 Å had been shown by Wyckoff (1920*b*). In an examination of the behaviour of X-ray tubes and photographic plates, he concluded 'the most useful Laue photographs are obtained by operating a tungsten tube, if this is used, at 50 kV'. He noted that the response of a photographic plate dropped off sharply as soon as the wave length exceeded 0.49 Å, the absorption edge for the silver in the emulsion. On the other hand, to avoid confusion of first and second order reflections, the main data should come from reflections with wave lengths between $\lambda_{\min}$ and $2\lambda_{\min}$. A Coolidge tube with tungsten target operated at 50 kV conveniently has $\lambda_{\min}$ at 0.24 Å and maximum intensity in its white radiation near 0.48 Å. At this voltage the K lines are not excited, while the L lines are beyond 1 Å.

The present generation of synchrotron Laue crystallographers will discern that such problems as optimum source characteristics, detector response, avoidance of multiple orders, wavelength normalization, optimum crystal setting and pattern interpretation had already been encountered by the pioneers of 70 years ago.

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