Laue crystallography

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Laue diffraction patterns from single crystals are now being recorded within a fraction of a second using highly intense synchrotron X-radiation. Apart from speeding up the data collection process, this remarkable reduction in exposure time permits the analysis of shortlived structural states formed during reactions. Recent developments in Laue crystallography and its application to various problems are presented.

THE landmark experiment conducted in 1912 by Friedrich and Knipping under the guidance of von Laue[1] at the University of Munich opened up an entirely new discipline of physics, X-ray crystallography. They recorded, for the first time, the diffraction image of a crystal of copper sulphate using polychromatic or white X-radiation. Laue not only demonstrated the wave nature of X-rays but by extending these experiments to other crystals also he could correlate the diffracted spots to the arrangement of atoms in the crystal. Very soon Bragg's law[2] was formulated which relates accurately the spacing between atomic planes in the crystal and their orientation to the wavelength of the incident beam, when diffraction occurs. Since then, crystal structures of several thousand molecules have been solved using the intensities of diffracted rays from single crystals using single wavelength characteristic X-rays. The ease in interpreting the diffraction patterns using such monochromatic radiation and the nonavailability of intense white radiation forced Laue method into oblivion for several decades.

Photographing Laue diffraction patterns in 1984 of the crystals of pea lectin (Figure 1) by Helliwell[3], and of bovine intestinal calcium-binding protein and of horse methemoglobin by Moffat *et al.*[4] using synchrotron white X-radiation was a great step forward in macromolecular crystallography. This reintroduction of Laue crystallography was possible because of the developments in synchrotron technology aimed at obtaining highly intense white radiation by installing special insertion devices called the undulators and wigglers between the bending magnets of the storage ring. The exposure times were only a few seconds. Since a wavelength range (0.1–4.0 Å) is used, several lattice planes diffract simultaneously selecting the appropriate wavelength even when the crystal is stationary. As a result, a spectacular panorama of spots appear on each film. The information obtained in such short exposures is enormous as a large portion of the reciprocal space is represented on the photographs in each setting. An old method was revived, a new tool was discovered paving way for what was to become kinetic crystallography or the time-resolved crystallography. While the advances in computer technology helped in predicting and deciphering the complicated Laue patterns accurately and in processing and measuring intensities of the diffraction spots on the photographs, developments in biochemistry and chemistry contributed a great deal towards applying Laue method to a better understanding of biological reactions.

Precise determination of three-dimensional structures of several biological molecules has enhanced our knowledge of structure–function relations and information inferred from the structures has helped to understand several biological processes at the atomic level. Synchrotron monochromatic radiation played a key role in speeding up the data collection procedure for macromolecules. Still,

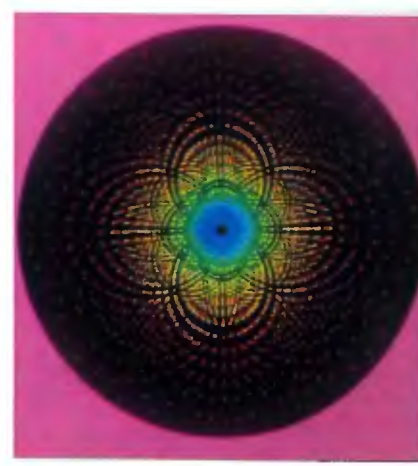

Figure 1. Simulated Laue diffraction pattern from a crystal of pea lectin (orthorhombic, $a = 50.73$, $b = 61.16$, $c = 136.59$Å with c axis along the X-ray beam, **a** axis horizontal and **b** axis vertical) in the resolution range 100 – 4 Å. Different colours represent different wavelength ranges (0.2 to 4 Å) of the incident X-ray beam. Blue represents 0.2 Å while red represents 4 Å.

using conventional techniques, it is not possible to investigate changes that happen in a fraction of a second or less as is the case with most reactions in biological systems. The Laue method permits studies on such dynamic events as large amounts of data can be collected extremely rapidly, setting an entirely new direction for X-ray crystallography. It is now possible to obtain structural information on the shortlived intermediates and subtle conformational changes in the proteins that occur during reactions. Direct visualization of a reaction could be achieved by taking a series of snap shots during the course of the reaction. Phase transitions and structural changes due to variation in temperature and other parameters could be studied. The process of substrate binding to enzymes could be followed to study how the end product is formed. In the past decade Laue method has been applied to tackle several such problems, resulting in many outstanding contributions, some of which are discussed here.

Johnson and her colleagues[5] reported the first successful attempts to demonstrate Laue method as a suitable tool to investigate reaction mechanisms by binding maltoheptose, a polysaccharide substrate, to glycogen phosphorylase b. By diffusing maltoheptose into a crystal of the enzyme, they recorded, for the first time, the diffraction pattern when the reaction was actually taking place. Using only one crystal, Laue photographs were taken before, during and after ligand binding to the enzyme, the exposure time being 1 second at each stage. Difference Fourier maps calculated using data from the first and the third pictures, i.e. between the native and the complex structures clearly showed the bound polysaccharide and movements in the side chains involved in substrate binding. However, diffraction pattern during the reaction was diffuse, indicating structural disorder. These elegant experiments confirmed the feasibility of using Laue method for structural investigations of fast phenomena in single crystals.

The credit for capturing a transient intermediate during the actual catalysis goes to Schlichting *et al.*,[6] who investigated the hydrolytic pathway of the p21 protein with the help of 'caged compounds'. No mimics for the ligand were used, nor any artificial means to slow down the reaction sought. The structure of a true intermediate in a real catalytic reaction was determined. The H-ras oncogene protein product p21 exists in an active form when complexed with GTP. This is a short-lived complex as hydrolysis of GTP to GDP takes place due to the GTPase action of p21. A stable form of this complex was formed by crystallizing p21 with a photolabile GTP derivative or the 'caged GTP', in which the nucleotide was made inactive by a photosensitive group. Flashing of light from a xenon lamp at the crystal caused removal of the protecting group and hydrolysis was initiated. Laue photographs taken four minutes later with 10–15 seconds exposure revealed the structure of the p21 + GTP complex.

Another example of direct visualization of intermediates is the light-triggered binding of pyrone to chymotrypsin.[7]. Unlike the previous example, in this experiment, the enzyme itself was inhibited with a covalently linked photodissociable group. Laue patterns obtained at different stages of the reaction with 5 second exposure revealed the structures of (i) this complex before irradiation of the crystal, (ii) free enzyme after irradiation of the crystal, and (iii) the complex of pyrone with chymotrypsin several hours after photolysis. During photolysis the structure seemed to have been disordered.

In one of the recent investigations[8], Laue method was used to locate the water molecule responsible for hydrolysis in trypsin. Crystals of a transiently stable intermediate *p*-guanidinobenzoyl (GB) trypsin were prepared. The GB group was released by a pH jump and Laue photographs before, immediately after the pH was raised from 5.5 to 8.5 and 90 minutes later, were recorded. The difference maps revealed a water molecule in the high pH form in a position suitable to attack the scissile bond and a gradual diminishing of the GB group density as the reaction progressed.

Studies on several other aspects of macromolecules were initiated soon after the potential of the Laue method for real time study of structural changes was discovered. Thermally induced changes in lysozyme were observed[9] by moving the film during the experiment as the temperature varied. Each spot appeared as a streak of varying intensity, suggesting the possibility of using Laue technique to obtain answers for the problem of protein folding. The successful determination of the positions of the Eu atoms using data from Laue pictures of native and heavy atom derivatives of xylose isomerase[10] indicated that this method can be used to solve protein structures. The phase transition from 4 Zn to 2 Zn was recorded in insulin[11]. The following studies confirmed the capacity of the Laue method to yield accurate results: Data were collected on the crystals of bovine pancreatic trypsin and the structure was refined successfully.[12] Binding studies on carbonic anhydrase II were performed[13] which confirmed the tetracoordination of Zn and revealed small structural changes.

The only application of Laue technique to virus crystals was the determination of metal-binding sites in tomato bushy stunt virus[14]. About 90% of the data between 6 and 3.5 Å resolution could be obtained from a single exposure of 24 seconds.

High quality Laue pictures were obtained[15] exposing a microcrystal of Gramicidin A ($30 \times 35 \times 10\ \mu m^3$) for 50 seconds showing how this technique allows structure determination even if crystallization attempts fail to yield large crystals.

Although work on macromolecules dominated this field, there were a few reports on small molecules. The first structure of a small molecule to be refined using the synchrotron white X-radiation was α-$AlPO_4$ (berlinite)[16]

The final positional parameters were found to be in good agreement with those obtained using diffractometer data. Laue photographs were also used to determine the structures of two organometallic compounds: $Rh_6(CO)_{14}(Ph_2PCH_2PPh_2)$ (ref. 17) and $FeRhCl(CO)_5Ph_2PCH(=CH_2)PPh_2$ (ref. 18) by Patterson and Fourier methods. It was demonstrated by structure refinement[19] and locating the hydrogen atom positions[20] in small molecules that Laue data compare well with data from monochromatic radiations. Direct methods were employed to solve the structures in both these examples. The time taken for the entire experiment on each of these small molecules was only a few seconds. The shortest exposure times were obtained by Szebenyi *et al.*[21] using synchrotron X-radiation emitted by single bunch electrons. They recorded 120 picosecond Laue patterns of an alkaloid and of tetragonal hen egg white lysozyme crystals and showed that these data compared well with monochromatic data.

The possibility of conducting Laue experiments without synchrotron radiation has been explored using white X-rays from sealed tubes for an organic molecule[22] and for hen egg white lysozyme[23].

The Laue technique offers many advantages over conventional X-ray methods. It can be applied to both small and large molecules. The time required for data collection ranges from picoseconds to seconds, compared to hours or days using monochromatic radiation. Integrated intensities of several reflections can be obtained from single diffraction pictures of a stationary crystal. However, it has a few limitations; the main drawback being the loss of a large number of reflections due to spatial and energy overlaps. Spatial overlaps occur because of the closeness of the spots on Laue pictures especially at low resolution and for crystals of very large unit cells. Energy or harmonic overlaps are caused when directions of diffracted rays from different reciprocal planes coincide. Advances in the theoretical front have mainly focussed on obtaining as much data as possible from minimum number of exposures. A detailed analysis of the distribution of the overlapping reflections was given by Cruickshank *et al.*[24, 25]. To fully exploit the high speed of the Laue method, strategies for data collection and optimal orientations of the crystal for the 11 Laue classes were discussed[26]. Another complication arising from the use of multiwavelength radiation is the dependence of the corrections applied in data reduction on the wavelength. Even though there were a few earlier attempts[9,16], the wavelength normalization incorporated in the processing software developed by Helliwell *et al.*[27] based on symmetry equivalents is being used widely. This package also has a procedure to separate harmonic doublets using data from multi-film packs. An algorithm by Shrive *et al.*[28] to deconvolute spatial overlaps has been successfully used[14] Methods based on gnomonic projections were developed for estimating the limits on the resolution and wavelength[29] which were required for the determination of cell parameters from single Laue pictures[30]. A strategy combining monochromatic and Laue techniques has been described[31] for optimizing experimental parameters and for reliable prediction of Laue patterns.

Lack of completeness of data remains still a major weakness of this method. This effect is more pronounced in the lower resolution shells affecting the quality of the electron density maps[12]. Unlike the monochromatic method, the Laue method is extremely sensitive to disorder in crystals. Even though radiation damage is a more serious problem when polychromatic radiation is used, significant damage is not noticed in Laue diffraction experiments as the exposure times are extremely short.

A number of reviews on the applications of Laue crystallography have appeared from time to time[32–35]. The latest developments in this field were presented in a discussion meeting at the Royal Society in London, the proceedings of which were published last year[36]. The most recent review article by Johnson[35] is a consolidated report of these studies. The meeting highlights the following new and emerging aspects of time-resolved crystallography: (i) combination of cryoenzymology and Laue crystallography, (ii) toast rack technique for better deconvolution of spatial overlaps and (iii) charge-coupled devices for real time viewing of Laue patterns. With these new advances, X-ray crystallography that has been considered to be capable of providing information only on static phenomena until now, has been elevated to a dynamic one, making four-dimensional space–time observation of biological processes and structures possible.

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A new look at the endocrine regulation of egg maturation in the decapod crustaceans

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Female reproduction, especially the egg maturation in the ovary of decapod crustaceans, is negatively controlled by an inhibitory neuropeptide from X-organ /sinus gland complex. Recent structural studies reveal that its primary structure is related to that of other eye stalk peptides such as crustacean hyperglycemic hormone and molt-inhibitory hormone. This peptide seems to inhibit yolk synthesis and uptake into the oocytes. Information on a gonad stimulatory hormone, purportedly originating from the neuro-secretory cells of brain and thoracic ganglia, is still very preliminary. Recent evidence indicates that the methyl farnesoate, a sesquiterpenoid compound synthesized by the glandular mandibular organ, could stimulate egg maturation, in much the same way the corpora allata controls oogenesis in insects. Application potentials of endocrine manipulation to crustacean aquaculture are discussed.

MOST decapod crustaceans lay a large number of yolk-rich eggs which contain a high-density lipoprotein as the main yolk protein, often conjugated to carotenoid pigments[1–3]. Both structural and biochemical evidences indicate a biphasic vitellogenesis in Crustacea, with the ovary initiating the biosynthesis of yolk within the growing oocytes, followed by a selective sequestration of yolk precursor molecule from the haemolymph (see Adiyodi and Subramoniam[4]). However, divergent views have been expressed regarding the ultimate site of yolk precursor synthesis in the extra-ovarial sites. Equally unsettled is the problem of endocrine regulation of egg maturation in crustaceans. However, by the recent upsurge in crustacean aquaculture, endocrine manipulation of reproductive phenomena has gained new impetus all over the world. In this article we review the very recent progress made in the hormonal control of vitellogenesis in decapod crustaceans.