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Introductory overview

Louise N. Johnson[a] and Tom L. Blundell[b]

[a]*Laboratory of Molecular Biophysics and Oxford Centre for Molecular Sciences, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK, and* [b]*Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, UK*

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The determination of the three-dimensional structure of the F1 ATPase by Jan-Pieter Abrahams, Andrew Leslie, Rene Lutter and John Walker (Abrahams *et al.*, 1994) at the MRC Laboratory of Molecular Biology, Cambridge, has illustrated the power of crystallography in defining the three-dimensional structures of complex macromolecular assemblies. Such assemblies, usually involving several components, appear to regulate most cellular processes, including membrane receptor responses, redox processes, cell signalling, the cell cycle, DNA replication, transcription and translation. Large assemblies also mediate many of the architectural features of living systems, including the cell cytoskeleton and viral capsids. Most of these systems are dynamic; indeed, many are transient, allowing regulation of cellular systems.

However, crystals of large unstable complexes are not easily studied in-house. Conventional sources provide X-rays of insufficient intensity, poor collimation and single wavelength. Synchrotron X-rays have none of these shortcomings; they can provide superb X-ray diffraction patterns from huge and complex molecules and they can even be used to investigate dynamic systems. It is not surprising that they are now in demand, the world over (Hasnain *et al.*, 1994), to provide X-rays for macromolecular diffraction experiments. This issue draws together a series of papers that highlight the achievements and advances of synchrotron radiation research for structural biology. The editors of the *Journal of Synchrotron Radiation* are congratulated for an excellent volume in which individual authors have contributed thoughtful analyses to both the historical development of the field and a description of the experimental protocols that have made the biological triumphs possible.

Synchrotron radiation has transformed macromolecular crystallography. When we were engaged 25 years ago in writing a monograph on protein crystallography (Blundell & Johnson, 1976), synchrotron radiation research for structural biology was in its infancy. Pioneering experiments on muscle had been carried out by Rosenbaum *et al.* (1971) at DESY, Hamburg, and preliminary protein crystallography results obtained at the DCI storage ring at LURE (Orsay), NINA (Daresbury) and at SPEAR (Stanford). These experiments were carried out in parasitic mode using first-generation synchrotron sources designed for particle physics. In the UK, Daresbury had just begun to recruit crystallographers from our community to contribute to the design of stations at a second-generation synchrotron. The opening of the dedicated radiation source at SRS, Daresbury, in 1981 gave rise to a new chapter for research.

When second-generation synchrotrons were up and running, many of us collected X-ray data from crystals of intermediate complexity and successfully produced high-resolution analyses. We even tried anomalous dispersion experiments and Laue diffraction. The power of these sources is demonstrated by the account of virus structures solved [Rossmann (1999); this issue] where the non-crystallographic symmetry exhibited by the icosahedral viruses provided a powerful method for phasing, allowing bootstrapping from low-resolution phases. But technical difficulties still needed to be overcome and the methods developed. This volume reviews the development of methods for phasing, especially multiple anomalous dispersion (MAD) [see Cassetta *et al.* (1999) and Fourme *et al.* (1999); this issue, where the articles also illustrate the beneficial interactions and sharing of information between scientists at different synchrotron sources]. The breakthroughs came from a careful attention to theory and a real focus on experimental stations. MAD has become a general approach with the ability to introduce seleno-methionine into proteins using recombinant techniques [see Hendrickson (1999); this issue].

Metalloproteins constitute nearly 30% of all known proteins. Small shifts of metals and their ligands in proteins can have significant effects for biology (for example, the 0.5 Å movement of the heam iron in heamoglobin on binding oxygen that triggers the allosteric response). The continuous spectra of synchrotron radiation allows the analysis of the absorption edges of metals in proteins, a requirement for use of anomalous dispersion data, and which has led to the development of the X-ray absorption fine structure (XAFS) technique that can provide precise information on oxidation and coordination state of metals in proteins [see Hasnain & Hodgson (1999); this issue].

However, third-generation synchrotron sources, such as ESRF, were needed to realise our wilder dreams! The determination of the transcriptionally active core particle

of the bluetongue virus, a molecular assembly with a diameter of 700 Å, could not have been achieved on a second-generation source and required meticulous attention to beamline optics [see Diprose *et al.* (1999); this issue]. The intensity at the beam at ESRF allowed measurement of many millions of weak reflections, in a short time, and the parallel beam ensured their resolution. Similar advantages of third-generation synchrotrons were found in fibre experiments as illustrated by Wakabayashi & Yagi (1999) (this issue) at SPring-8 for diffraction peaks on the meridian in the diffraction pattern of the muscle sarcomere. There are, of course, other advantages of synchrotron radiation; the variation in wavelength allows less-damaging shorter-wavelength radiation to be used and the reduction of background allows data of better quality to be collected, often enhancing the resolution.

The high intensity of synchrotron radiation also allows data to be collected on unstable intermediates, even for complexes such as the rhinovirus [see Rossmann (1999); this issue]. Transient structures – stable for less than 1 ms – can be observed in contracting muscle fibres. But perhaps the best hope for time-resolved studies is the use of Laue methods. The major problems here have proved to be obtaining synchrony in the reaction process between the different molecules within the crystals and in identifying a system where interesting changes can take place within the confines of the crystal lattice. The article by Ren *et al.* (1999) in this issue summarizes the advances that have been made, both in experimental design and data-processing software, and describes the application of the Laue method to nine different systems where the results have been informative. Exposure times as short as 100 ps are achievable but, even at third-generation synchrotrons, intensity restrictions limit the application of such ultrafast experiments to reversible reactions where multiple exposures can be recorded at identical points as the reaction progresses through the cycle.

The challenges for the future include obtaining higher fluxes and faster detectors; these are undergoing rapid improvement. Many of the problems will remain biological. Producing suitable crystals, diffracting to high resolution, has proved the rate-determining step in both membrane [Tsukihara & Lee (1999); this issue] and ribosomal proteins [Schlunzen *et al.* (1999); this issue], but fantastic progress has been made here also. There are now more than 18 membrane protein structures solved by X-ray and electron microscopy methods, including bacterial and plant proteins involved in solar energy conversion, proteins involved in respiratory chain bio-energetic systems, and channel-forming proteins. The structure of the membrane cytochrome C oxidase, a 13-chain complex of the respiratory chain, defined by Tsukihara in Japan, has been a dramatic achievement. The competition for the first structure of a ribosome has been joined by several groups in Europe and more recently the USA. Already, medium-resolution diffraction data are being collected. The challenges here have been to use biology to produce a ribosome complex that is sufficiently robust for crystallization (a bacterial source from the Dead Sea provided a breakthrough) and to develop methods of heavy-atom clusters, molecular markers, and incorporation of data from other sources, especially electron microscopy, to solve the phase problem where there is no help from non-crystallographic symmetry.

The biological challenges will not only be in size and complexity but also in trapping unstable short-lived complexes that exist for only minutes in the cell. These include signalling complexes, where weak binary interactions cooperatively contribute to tight but often transient multi-component systems, and DNA replication, where transient assemblies of polymerases, clamps *etc.* mediate replication. Synchrotron radiation is sure to play a key and enabling role. We should see more Nobel laureates in the future who, like John Walker and his colleagues, have thoughtfully exploited synchrotron X-rays to advance science at the interface between molecular and cell biology.

Now biological problems are often the driving force for the next generation of synchrotrons. Medical and biological agencies are key to their funding as has been shown by the imaginative initiative of the Wellcome Trust in the UK to contribute in a major way to the new Diamond synchrotron. At DESY, a 500 GeV colliding-beam TESLA project plans to produce a free-electron laser (FEL) of breathtaking brilliance for 1 Å X-rays (about ten orders of magnitude higher peak brightness than currently available from third-generation sources). A pilot FEL for VUV and the soft X-ray region should become operational for the year 2000 but the jump from VUV to the hard X-ray region is formidable. A totally new science has to be explored. What possibilities could this open for structural biology?

With the extremely bright source it may be possible to escape the benevolent tyranny of the crystal and record molecular transforms from individual molecules. Studies by Miao, Charalambous, Kirz & Sayre (private communication) have shown that the soft-X-ray molecular transform of a micrometre-size non-crystalline specimen can be inverted to form an image in which the phase problem is overcome by over-sampling the diffraction pattern and use of an iterative algorithm. In order to record a molecular transform, would the molecule need to be tethered in order to localize it sufficiently or would a spray technique prove possible? How can a reasonably conformationally homogeneous population of molecules be generated or would structural differences be discernable from transforms following the methods developed for electron-microscopy single-particle image reconstruction? Finally, will the biological molecule withstand such a bright beam? Will cooling with helium to 30 K alleviate radiation damage? Cooling to such temperature did not prove effective in studies with the ribosome. There are challenges here for the physicist and the biologist. With more beamlines for macromolecular crystallography available and increases in the brilliance of sources, the next 25 years should be as equally exciting as the last 25 years. Synchrotrons will need to be integrated into biological science communities as well

as having access to first-rate physics, engineering and design.

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