Synchrotron Radiation and Crystallography: the First 50 Years

J. R. HELLIWELL

Department of Chemistry, University of Manchester, Manchester M13 9PL, England.
E-mail: john.helliwell@man.ac.uk

Abstract

Synchrotron radiation (SR) was first seen in the laboratory some 50 years ago. The properties of SR for X-ray crystallography became recognized and harnessed as synchrotrons with the requisite machine energies became available. SR source characteristics and operation have increasingly become tailored to SR applications as the field of SR research has matured from its beginnings in high-energy physics laboratories such as Frascati, DESY in Hamburg, SPEAR in Stanford and NINA in Daresbury. SR sources, beamline optics and detectors have considerably improved in specification and performance especially over the last two decades and methods have also evolved. A diverse range of applications of SR in crystallography, and cognate techniques, has been stimulated. Much scientific research in physics, chemistry, biochemistry, biology and medicine utilizes SR in diverse ways. World-wide there

Professor J. R. Helliwell BA (Physics, York), DPhil (Molecular Biophysics, Oxford), DSc (Physics, York), FInstP, FRSC, FIBiol. John Helliwell worked at Daresbury Laboratory's Synchrotron Radiation Source (SRS) from 1979 to 1993, whilst also a Joint Appointee with the Universities of Keele, York and Manchester, and as a scientific civil servant, and earlier on the NINA synchrotron. He has served on the ESRF Science Advisory Committee as Vice-Chairman and then Chairman, on the ESRF Machine Advisory Committee, and on the Council of the ESRF; as Chairman of the UK SRS Panel for Protein Crystallography; as Chairman of the Cornell University MACCHESS Advisory Committee; as a Member of the Elettra Sincrotrone Trieste Review Committee; as a Reviewer of the EMBL Outstation in Hamburg; on the Advisory Committee of the Georgia SER-CAT and a consultant to the Industrial Macromolecular Crystallography Association's CAT, both at APS. He was the founding Chairman of the International Union of Crystallography's Commission on Synchrotron Radiation. He is Professor of Structural Chemistry of the University of Manchester, a founding Editor of the Journal of Synchrotron Radiation, an Editor of the OUP Book Series on SR and Editor-in-Chief of Acta Crystallographica.

are now many dedicated facilities for SR and, for those emitting into the X-ray region of the electromagnetic spectrum, crystallography is a major user. Exciting new scientific opportunities now beckon.

1. Introduction and early history

It is a happy and remarkable coincidence that it was around the same time that synchrotron radiation was first seen (in 1947 by Elder *et al.*, 1947, 1948) that the IUCr and its first journal *Acta Crystallographica* were launched in 1948. However, it was not until the 1960s, 1970s and 1980s that the properties of SR were harnessed for X-ray crystallography and cognate techniques, such as noncrystalline diffraction and X-ray absorption spectroscopy, as synchrotrons and storage rings with the requisite machine energies became available. A special issue of the IUCr's *Journal of Synchrotron Radiation* was published in November 1997 to mark the first 50 years of SR.

In a very perceptive paper, Parratt (1959), based at Cornell, described the potential use of "synchrotron orbit-radiation" in the "large realms of X-ray structure analysis and in X-ray absorption spectroscopy". Parratt assessed the relative merits of "6 BeV SR and 1.07 BeV SR *versus* a Cu $K\alpha$ 1 kW X-ray tube" concluding that a "6 BeV beam at 1 Å is enormously advantageous" and that "much greater intensity would be produced by applying a sudden high magnetic field pulse in a small length of the path of the high-energy electron... (which) could be performed as a 'kink' in the synchrotron orbit, or at the output of a linear accelerator". Parratt also commented on "the radiating time of one 'bunch' of electrons as it passes in orbit, the maximum rate for useful irradiation of material with the 6 BeV beam is about 10^{20} photons per second at 1 Å". At the time, a "machine (was) in construction at Cambridge at 6 BeV".

The first SR X-ray spectra were recorded by Cauchois *et al.* (1963) using the Frascati synchrotron functioning at 1.1 GeV. SR soft X-rays between 5 and 14 Å were used to record the *K*-absorption spectrum of aluminium and the L_{III} edge of copper on photographic film. This study also represented the first use of crystalline diffraction, *i.e.* involving a quartz crystal and the struc-

ture factor for the $10\bar{1}0$ reflection. The circulating current comprised 10^9 electrons in orbit. Cauchois *et al.* (1963) conclude with the remark that the work opens up interesting prospects!

The X-ray diffraction experiment of Rosenbaum *et al.* (1971) on muscle fibres at the 7.2 GeV synchrotron at DESY, Hamburg was a very important practical demonstration involving the comparative intensity of two muscle fibre diffraction patterns (Fig. 1) which showed that the DESY synchrotron (at 5 GeV) was about ten times more effective than one of the most intense conventional X-ray sources available then.

The physics of the SR source spectral emission predicted by Iwanenko & Pomeranchuk (1944) and Blewett (1946) was fully described by Schwinger (1949) which was 'universal' to all machines of this type, *i.e.* wherever charged particles such as electrons (or positrons) travelled in a curved orbit under the influence of a magnetic field, subject thereby to centripetal acceleration. At a speed very near to the speed of light, the relativistic particle emission is concentrated into a tight forward-radiation cone angle. There is a continuum of Doppler-shifted frequencies from the orbital frequency up to a cut-off. The radiation is also essentially plane polarized in the orbit plane. In high-energy-physics machines, the particle beam, for target or colliding beam experiments, would be somewhat unstable however, and thus, whilst pioneering SR experiments ensued through the 1970s, a considerable appetite was stimulated for storage rings with stable source position, for fine focusing onto small samples such as crystals, and with a long beam lifetime, and machines dedicated to SR for more challenging and extensive data collection. Crystallography has been both an instigator and major beneficiary of these developments through the 1970s and 1980s onwards. The evolution of new machines and the massive increase in source brilliance, year on year,

are shown in Fig. 2. The most recent machines to come on-line are SPring-8 (8 GeV) and MAX2 (1.5 GeV), thus illustrating the need for a range of machine energies today. An example of a machine lattice (SRS at Daresbury, the first dedicated storage-ring X-ray source) is shown in Fig. 3.

2. Principles of SR

The properties of synchrotron radiation can be described in terms of the following:

$$\text{flux} = \text{photons s}^{-1} (0.1\% \, \delta\lambda/\lambda)^{-1} \qquad (1a)$$

$$\text{brightness} = \text{photons s}^{-1} (0.1\% \, \delta\lambda/\lambda)^{-1} \, \text{mrad}^{-2} \qquad (1b)$$

$$\text{brilliance} = \text{photons s}^{-1} (0.1\% \, \delta\lambda/\lambda)^{-1} \, \text{mrad}^{-2} \, \text{mm}^{-2}. \qquad (1c)$$

The mrad^2 refers to radiation solid angle delivered from the source and mm^2 the source cross-sectional area.

The machine emittance ε is the product of the divergence angle, σ', and the source size, σ:

$$\varepsilon = \sigma\sigma'. \qquad (2)$$

The horizontal and vertical emittances need to be considered separately.

Multipole magnet devices can be placed (inserted) in straight sections of the synchrotron or storage ring. They can be designed purposely to enhance specific characteristics of SR, namely:

(*a*) to extend the spectral range to shorter wavelengths (superconducting wavelength shifter as seen in Fig. 3);

(*b*) to increase the available intensity (multipole wiggler);

(*c*) to increase the brilliance *via* interference and also yield a quasi-monochromatic beam (undulator);
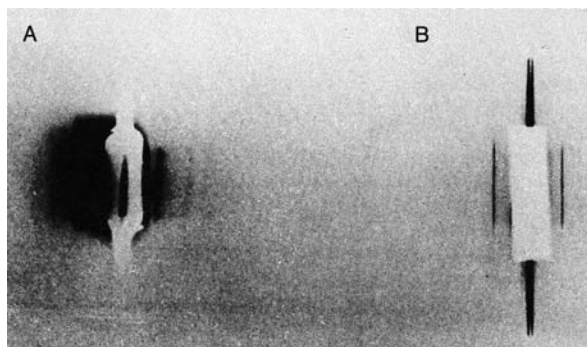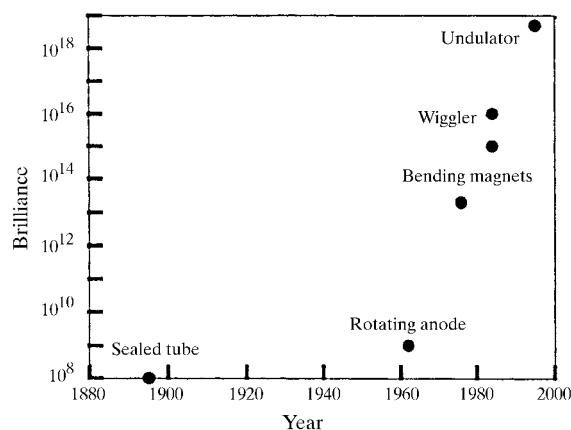

Fig. 1. The first X-ray biological diffraction experiment was conducted at DESY in Hamburg. The equatorial reflections from the dorsolongitudinal flight muscle of *Lethocerus maximus* recorded with: (*A*) the monochromated SR beam, electron energy 5 GeV, beam current 8 mA, exposure time 15 min; (*B*) Elliott fine-focus rotating anode tube at 40 kV, 15 mA, exposure time 1 h. Reprinted with permission from *Nature* (Rosenbaum *et al.*, 1971). Copyright (1971) McMillan Magazines Ltd.

Fig. 2. Schematic of the evolution of X-ray source brilliance [photons s^{-1} mrad^{-2} mm^{-2} (0.1% $\delta\lambda/\lambda$)$^{-1}$] in the hundred years since Röntgen's discovery of X-rays in 1895. Adapted from Coppens (1992).

(*d*) to provide a different polarization (*e.g.* rotate the plane of polarization, produce circularly polarized light *etc.*).

The classification of a periodic magnet ID as an undulator is based on whether the angular deflection, δ, of the electron beam is small enough to allow interference between the radiation emitted from one pole to interfere directly with that of the next pole. The angular deflection δ is changed by opening or closing the gap between the pole pieces. Short-wavelength undulator emission is the province of the new third-generation machines such as the ESRF in Grenoble, France (6 GeV), the APS at Argonne National Laboratory, Chicago, USA (7 GeV) and SPring-8 at Harima Science Garden City, Japan (8 GeV). Examples of SR spectral curves are shown in Fig. 4; Fig. 4(*b*) shows the distinctly different emission from an undulator.

3. At the sample

The sample acceptance, α, is a term that is useful as a direct analogue of the synchrotron machine emittance [equation (2)], *i.e.*

$$\alpha = x\eta, \tag{3}$$

where x is the sample size and η the mosaic spread. For example, if $x = 0.1$ mm and $\eta = 10^{-3}$ rad (0.057°) then $\alpha = 10^{-7}$ m rad or 100 nm rad.

At the sample position, the intensity of the beam, usually focused, is a useful parameter:

$$\text{intensity} = \text{photons s}^{-1} \text{ (focal spot area)}^{-1}. \tag{4}$$

Moreover, the horizontal and vertical convergence angles are ideally kept smaller than the mosaic spread, *e.g.* ≤ 1 mrad, so as to measure reflection intensities with optimal peak to background.

Fig. 3. Part of the machine lattice at SRS Daresbury. In the centre is the SRS superconducting wiggler 'wavelength shifter' at the time of its installation in 1981. Reproduced with the permission of Daresbury Laboratory.

To produce a focal-spot area that is approximately the size of a typical crystal (≤ 0.3 mm) and with a convergence angle ≤ 1 mrad sets a sample acceptance requirement to be met by the X-ray beam and machine emittance. A machine with an emittance that matches the acceptance of the sample greatly assists the simplicity and performance of the beamline (mirror and/or monochromator) optics design. The common beamline optics schemes are shown in Fig. 5.

In addition to the focal spot area and convergence angles, it is necessary to provide the appropriate spectral characteristics. In monochromatic applications, involving the rotating crystal diffraction geometry, a particular wavelength, λ, and narrow spectral bandwidth, $\delta\lambda/\lambda$, are used. Fig. 6 shows a white-beam broad-band Laue diffraction pattern from a protein crystal; the colour prediction beautifully illustrates the broad-band continuum of SR wavelengths striking the crystal sample.

4. Evolution of SR machines and experiments

The so-called first generation of SR machines were those that were parasitic on high-energy-physics operations
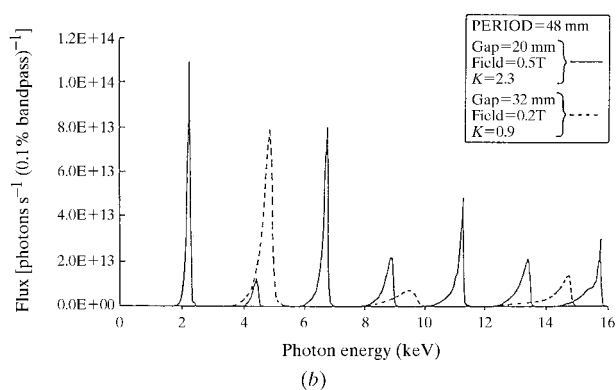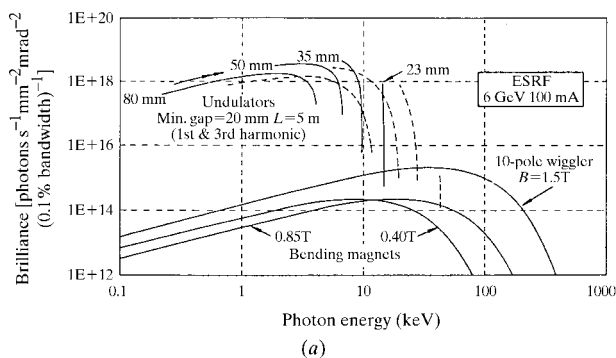

Fig. 4. SR spectra. (*a*) Brilliance of the different SR source types as exemplified by the different sources at ESRF (multipole wigglers and bending magnets and for the undulators the tuning range is indicated). (*b*) Example of an actual undulator emitted spectrum (ESRF). From Elleaume (1989). Brilliance values of 10^{20} photons s^{-1} (0.1% $\delta\lambda/\lambda)^{-1}$] mrad^{-2} mm^{-2} have now been reported from ESRF (see Elleaume *et al.*, 1998).

such as DESY in Hamburg, SPEAR in Stanford, NINA in Daresbury and VEPP in Novosibirsk. These machines had high fluxes into the hard X-ray range and enabled pioneering experiments, for example in protein crystallography, including multiple-wavelength anomalous dispersion (Phillips *et al.*, 1976; Webb *et al.*, 1977; Harmsen *et al.*, 1976; Helliwell, 1984), energy-dispersive diffraction (Bordas *et al.*, 1976, Buras & Gerward, 1975), EXAFS (Stern *et al.*, 1975), biological small-angle diffraction (Haslegrove *et al.*, 1977), DNA fibre diffraction (Skuratovskii *et al.*, 1978), and so on. Historical insights into the performances of these machines, from the current perspective, are described in detail for example by Huxley & Holmes (1997) at DESY, Munro (1997) at Daresbury and Doniach *et al.* (1997) at Stanford. A principal limitation was the problem of source movements, which degraded the focusing of the source onto a small crystal or single fibre and thereby the intrinsic brilliance of the beam (Haslegrove *et al.*, 1977). Some possible applications discussed were unfulfilled until brighter sources became available, *e.g.* nuclear scattering involving tiny SR bandwidths (Parak *et al.*, 1971; Mossbauer, 1975; Rüffer, 1992). Moreover, the two-wavelength crystallography phasing method of Okaya & Pepinsky (1956) and Hoppe & Jakubowski (1975), the three-wavelength method of Herzenberg & Lau (1967) and the algebraic method of Karle (1967, 1980) awaited more stable beams, which had to be

rapidly and easily tuneable over a fine bandpass ($< 10^{-3}$). Experiments to define the anomalous-dispersion coefficients, including dichroism effects, at a large number of wavelengths at an absorption edge in a variety of crystal structures, was conducted at SPEAR (Phillips *et al.*, 1978; Templeton & Templeton, 1985). Values of f' over a continuum of wavelengths in a real compound (Fig. 7) were explored in a profile approach (now called DAFS) by Arndt *et al.* (1982) at the newly commissioned SRS. Amongst the first structural results unique to synchrotron radiation was the site-specific identification of the manganese site in the protein pea lectin using two-wavelength anomalous-dispersion crystallography including the Mn K edge (Einspahr *et al.*, 1985).

The building of dedicated X-ray sources began with the SRS at Daresbury, which came on-line in 1980. Elsewhere in the world, building up their operation in the late 1970s and in the early 1980s were CHESS at Cornell, and under construction were the NSLS in Brookhaven and the Photon Factory (PF) in Japan. NSLS and PF came on-line in 1983 and 1984, respectively. There was thereby a rapid percentage increase in the number of operating machines and beamlines worldwide in the X-ray region for diffraction and X-ray absorption spectroscopy. The pioneering experiments continued and blossomed. Seminal work ensued in virus crystallography [Rossmann & Erickson (1983) at Hamburg and Daresbury and Usha *et al.* (1984) at LURE], Laue diffraction for time-resolved protein crystallography [Moffat *et al.* (1984) at CHESS and at
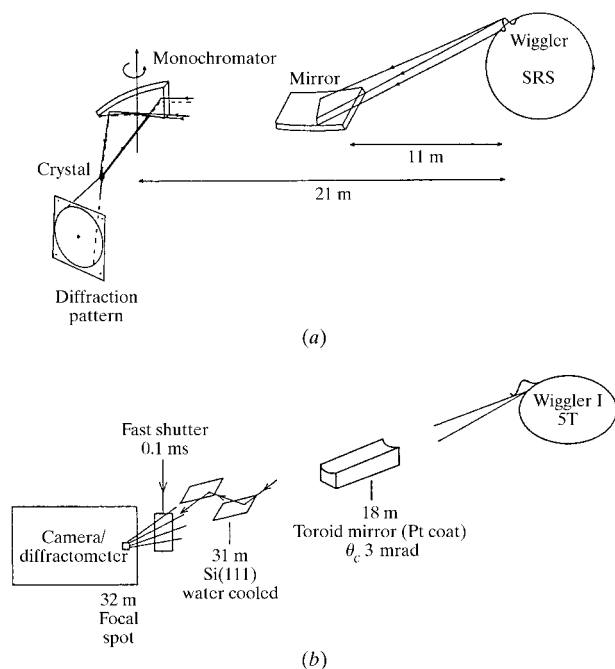

Fig. 5. Common beamline optics modes. (*a*) Horizontally focusing cylindrical monochromator and vertical focusing mirror (at station 9.6 at the SRS (Helliwell *et al.*, 1986). (*b*) Rapidly tunable double-crystal monochromator and point focusing toroid mirror [at station 9.5 at SRS (Brammer *et al.*, 1988)].
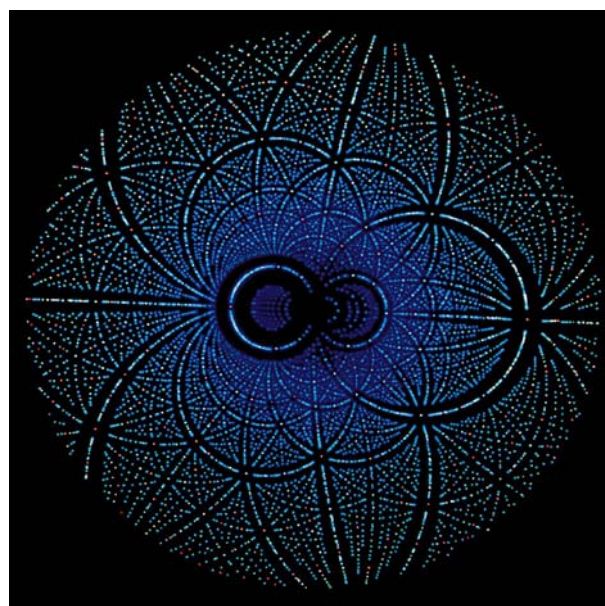

Fig. 6. Protein crystal Laue diffraction pattern (illuminating bandpass without monochromator, $\sim 0.4 < \lambda < 2.6$ Å) with the colour prediction using blue spots for short wavelengths through the 'rainbow colours' to longer wavelengths (Cruickshank *et al.*, 1991).

SRS, Helliwell (1984, 1985), Cruickshank *et al.* (1987, 1991), Hajdu *et al.* (1987*a,b*), Helliwell *et al.* (1989); also Bourenkov *et al.* (1996), Neutze & Hajdu (1997)]; multiple-wavelength anomalous dispersion (MAD) [Hendrickson *et al.* (1989) at SPEAR and PF, Guss *et al.* (1988) at SPEAR, Kahn *et al.* (1985) at LURE, Korszun (1987) at CHESS, Mukherjee *et al.* (1989) and Peterson *et al.* (1996) at SRS, Chayen *et al.*(1996) at ESRF, to name a few experiments]; diffuse scattering with SR [Doucet & Benoit (1987), Caspar *et al.* (1988), Glover *et al.* (1991)]; magnetic scattering [Brunel *et al.* (1983) at LURE]; dispersive EXAFS [Phizackerley *et al.* (1982) at SPEAR], and so on.

As early as 1979, discussions took place to plan a proposal for a high-brilliance insertion-device-driven European Synchrotron Radiation Source. A wide

Table 1. *A comparison of the final parameter list for the mature SRS, 1997, and the proposed upgraded machine, DIAMOND*

S/C = superconducting magnet; MW = multipole wiggler (permanent magnet design).

	SRS†	Diamond‡
Storage-ring energy	2 GeV	3 GeV
Circumference	96 m	350 m
Beam emittance	110 nm rad	15 nm rad
Beam current after injection	300 mA	300 mA
Typical dipole beam source sizes (σ)		
horizontal	900 µm	400 µm
vertical	200 µm	150 µm
Critical energy		
dipole	3.2 keV	20 keV (S/C)
wiggler	13.3 keV (S/C)	10 keV (MW)

† From Munro (1997).   ‡ From Suller (1994, 1998).

variety of discussion documents and workshops, and the ESR Project led by B. Buras and based in Geneva at CERN, culminated in the so-called 'Red Book' in 1987 *i.e.* the ESRF Foundation Phase Report (ESRF, 1987) totalling some 1000 pp. of machine, beamline and experimental specifications and costings. This then was the progenitor of the third-generation sources, characterized by their high energy and high brilliance, tailored to optimized undulator emission in the 1 Å range. Actually, the ESRF machine energy was initially set at 5 GeV but increased to 6 GeV to optimize the production of 14.4 keV photons to better match the nuclear scattering experiments, proposed initially by Mössbauer in 1975. The US proposed machine, the Advanced Photon Source at 7 GeV and the Japanese 8 GeV SPring-8 machine proposals followed, the higher machine energy enhancing the X-ray tuning range of undulators. Thus, spectroscopy and MAD tuning-based techniques would be facilitated with undulators on these machines whilst opening up studies involving ultra-small samples (of crystals, single fibres or tiny liquid aliquots) or very large unit cells. States of matter at much higher pressures could thus also be explored. Huge multi-macromolecular biological structures, of large viruses for example, would become accessible as well.

Today, a variety of enhanced national SR machines are being proposed and/or built. In the UK, the DIAMOND 3 GeV machine is proposed to replace the SRS, and likewise, in France, Soleil to replace LURE's machine. The SLS in Switzerland, as their first light source, is approved. The new sources are in essence characterized by high brilliance, *i.e.* low emittance. Already operational is the 2 GeV high-brilliance SR source ELETTRA in Trieste, the MAXII machine in Lund and the Brazilian Light Source in Campinas. In many ways, the national sources like SRS, LURE, DORIS and so on fuelled the case and specification for ESRF. Now the developments at ESRF, including high

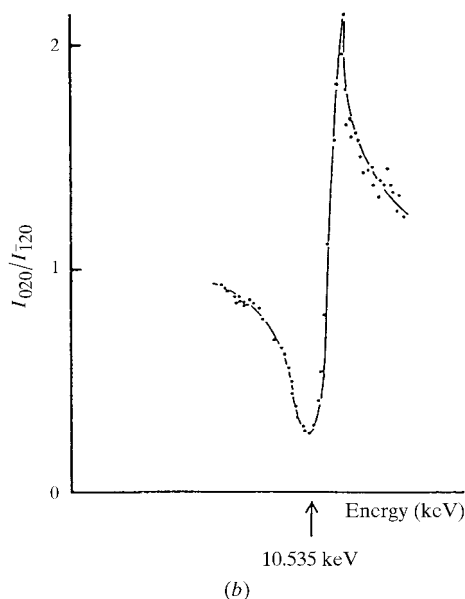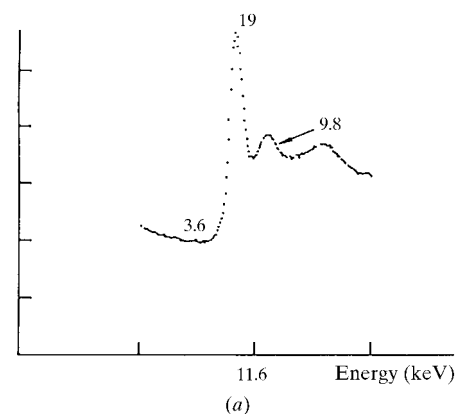
Fig. 7. Anomalous dispersion. (*a*) f'' as represented by an absorption spectrum [Pt L_3 edge as example, compound $K_2Pt(CN)_4$] (Helliwell, 1984). (*b*) f' as estimated by a continuous polychromatic profile method. Reproduced with permission from *Nature* (Arndt *et al.*, 1982). Copyright (1982) MacMillan Magazines Ltd.

harmonic emission of undulators *via* magnet shimming and narrow-gap undulator operation (Elleaume, 1998) are fuelling ideas and the specification of what is possible in these new national SR sources. Table 1 compares the parameters of the mature SRS of 1997 (from Munro, 1997) with the proposed design for DIAMOND (Suller, 1994).

In terms of evolution of X-ray sources, mention should be made of the X-ray free electron laser (XFEL); it now seems feasible that this will yield wavelength output well below the visible region of the electromagnetic spectrum. At DESY in Hamburg (Brinkmann *et al.*, 1997) and at SLAC in Stanford (Winick, 1995), such considerations and developments are being pursued, to provide a transversely fully coherent beam, pulse lengths of $\sim$200 fs (FWHM) and 8 to 10 orders of magnitude larger peak brilliance.

5. SR instrumentation

The divergent continuum of X-rays from the source must be intercepted by the sample cross-sectional area. The crystal sample acceptance, as seen above, is a good way to illustrate for the machine designer the sort of machine emittances required. Likewise, the beamline optics, mirrors and monochromators should not degrade the X-ray-beam quality. There are general instrumentation trends. Mirror surface and shape finish have improved a great deal in the last 20 years; slope errors of mirrors, even for difficult shapes like polished cylinders, which by bending produce a toroidal reflecting surface, are now around $1''$ for a length of 1 m, important for focusing over long distances. Choice of materials has evolved too from the relatively easy to work and finish fused quartz to silicon; silicon also has the advantageous property that at liquid-nitrogen temperature the expansion coefficient is zero (Bilderback, 1986). This has been of particular advantage in the cooling of silicon monochromators at the ESRF where the heat loading on optics is very high. An alternative approach with the rather small X-ray beams from undulators is the use of transparent monochromator crystals made of diamond, which is a robust material with the additional advantage of transparency thus allowing multiplexing of stations, one downstream from the other, fed by one straight section of one or more undulator designs. In this Special Issue, Hart & Berman (1998) review this subject in general; see also Helliwell (1992, ch. 5).

Detectors have been, and to a considerable extent are still, a major challenge. The early days of SR usage saw considerable reliance on photographic film for crystallography, as well as some usage of single-counter four-circle diffractometers. Evolution of area detectors in particular has been considerable and impressive, and in a variety of technologies. Gas detectors, *i.e.* the multiwire proportional chamber (MWPC), developed through various generations and types (Charpak, 1970;

Lewis, 1994; Fourme, 1997), represent the detector with the best detector quantum efficiency and capable of recording many simultaneous diffraction spots, but with limitations as to count rate (local and global) as well as restricted to use at wavelengths greater than $\sim$1 Å. The most popular devices and technologies for X-ray diffraction pattern data acquisition today are image plates (Miyahara *et al.*, 1986; Amemiya, 1997) and charge-coupled devices (CCDs) (Tate *et al.*, 1995; Allinson, 1994; Westbrook & Naday, 1997). Image plates and CCDs are complementary in their performances, especially with respect to their size and duty cycle; image plates being larger, *i.e.* with many resolution elements possible, but being slower to read out than CCDs. Both are capable of imaging well at shorter wavelengths than 1 Å and with high count rates. Both have overcome the tediousness of chemical development of film! Impressive performances for macromolecular crystallography are described for image plates (in a Weissenberg geometry) by Sakabe *et al.* (1995) and for CCDs by Tate *et al.* (1995).

An upcoming area-detector development is the so-called pixel detector. This is made of silicon cells each 'bump bonded' onto associated individual electronic readout chains. Each pixel has its own electronic counting chain and so is independently 'on-line'. Extremely high count rates are possible and large area arrays of resolution elements conceivable, at a cost. These devices then can combine the attributes of large image-plate-sensitive areas with the fast readout of CCDs, along with the count-rate capability and so on. Devices and prototypes are being pursued at Princeton/Cornell (Eikenberry *et al.*, 1998), Berkeley/San Diego (Beuville *et al.*, 1997), Imperial College, UK (Hall, 1995) and Oxford Instruments/Rutherford Appleton Laboratory (IMPACT detector programme).

6. Applications

There are a myriad number and range of applications and results from the use of SR in crystallography. The selection here is a glimpse of the results across physics, chemistry, biochemistry, biology and medicine.

6.1. *Applications in physics*

The special characteristics of SR have been exploited to explore high-pressure states of matter, surface physics, magnetic scattering and many more. The high brilliance of the beam allows small samples to be investigated, weak X-ray scattering cross sections to be stimulated, polarization effects to be investigated and the tunability to be used. Separately, in this Special Issue, is a detailed description of *n*-beam diffraction and phasing (Chang, 1998) and this will not be covered here. Complementary aspects of neutrons and SR in crystal-

lography, *e.g.* for high pressure and magnetic scattering, appear in an article in this Special Issue (Willis, 1998).

In high-pressure studies, the ability to obtain strong diffraction from smaller sample volumes has allowed an increase in the pressures attainable with diamond-anvil cells, DACs. For example, Akahama *et al.* (1995) explored the structural transition of oxygen at 96 GPa associated with a metallization transformation. This study involved loading liquid oxygen into a 150 μm diameter hole in a DAC at 77 K and the pressure increased up to a maximum of 116 GPa. X-ray diffraction data were recorded in angle-dispersive mode with $\lambda = 0.4817$ Å on ESRF BL3, with the 'wundulator' insertion device SR beam focused *via* a Bragg–Fresnel lens. It is a most interesting feature that a metal behaviour is exhibited by an element that is a gas at standard temperature and pressure!

Magnetic scattering has developed greatly with the application of SR, both in terms of the intensity to overcome a weak scattering cross section and also the tunability to yield resonance enhancement of the cross section. A recent book has been published covering this topic and applications (Lovesey & Collins, 1996) indicative of the large and growing activity in this field. The basic challenge is that the spin scattering amplitude, by which a light wave's magnetic field component is scattered, is reduced by a factor of $\sim 10^3$ to 10^4 times, compared with charge scattering of the electric field component and also most electrons in the sample are likely to be paired, which shrinks the weak signal still further. The first experimental observation of non-resonant magnetic diffraction was by de Bergevin & Brunel (1972), which was performed using a copper laboratory X-ray source, on NiO. Thereafter, Brunel *et al.* (1983) used SR to perform the first experimental observation of non-resonant magnetic diffraction on a powder of the ferrite $Zn_{0.5}Fe_{2.5}O_4$, undertaken at

LURE. In separate studies at the NSLS, strong (50-fold) resonance-enhanced magnetic diffraction was seen (Gibbs *et al.*, 1988) at the L_3 edge of Ho metal. A larger resonance enhancement was observed (McWhan *et al.*, 1990) at the M_4 edge in UAs leading to magnetic diffraction intensities of many thousands of counts s^{-1}, *i.e.* $\sim 1\%$ of the charge scattering, widening the possibilities still further. Recently, Lippert *et al.* (1994) at HASYLAB in Hamburg, using 80 keV photons, with their great penetration, studied MnF_2 at the (300) Bragg position. MnF_2 has a body-centred structure, in terms of charge scattering, and the associated body-centre absence condition is violated by the non-centred spin distribution of the Mn sites below the magnetization temperature.

6.2. *Applications in chemistry and biochemistry*

Many SR-based techniques are driving many new results in chemistry including chemical crystallography,
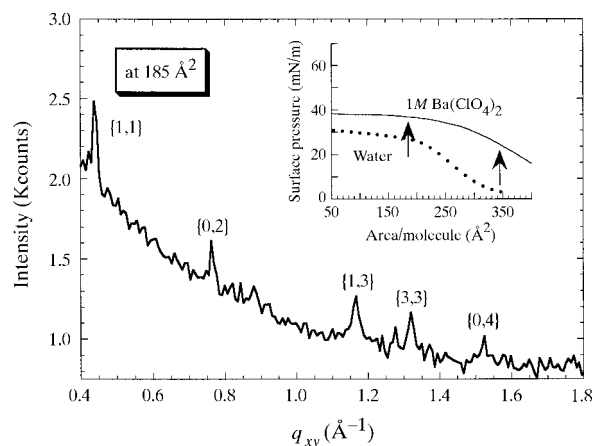
Fig. 9. Grazing-incidence liquid diffractometer investigation of molecular organization using DORIS. Reprinted with permission from Rapaport *et al.* (1997). Copyright (1997) American Chemical Society and with permission of the authors.

Fig. 10. The interface between chemistry, biochemistry and biology. Determination of the protonation states of carboxylic acid side chains in proteins *via* H atoms and resolved single- and double-bond lengths. After Deacon *et al.* (1997) using CHESS. Copyright Royal Society of Chemistry, UK.
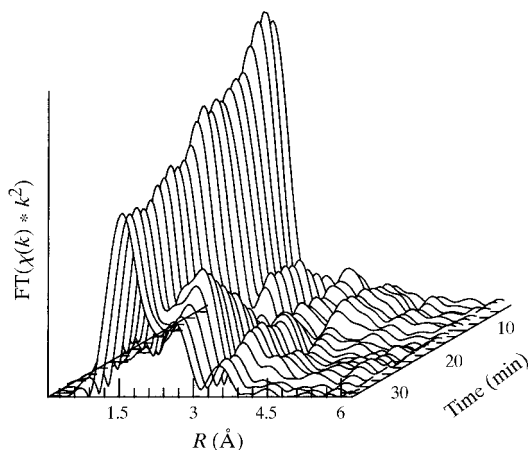
Fig. 8. Time-resolved EXAFS spectroscopy using dispersive geometry. Evolution of an *in situ* reaction with time. After Hagelstein *et al.* (1997) using ESRF (with permission of the authors).
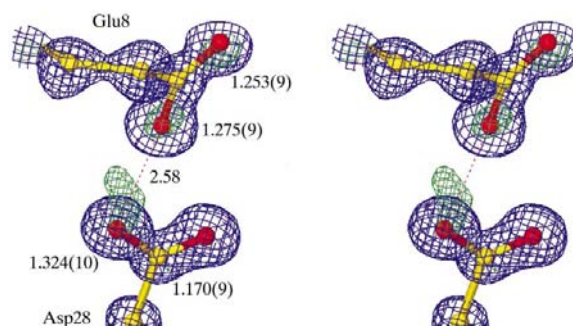
EXAFS/XANES, surface EXAFS and diffraction, and so on. An extensive survey of uses of SR in crystallography is given by Coppens (1992). A forerunner of small, and mosaic, chemical crystal structure data collection and analysis was the study of piperazine silicate using a TV area-detector diffractometer on the SRS wiggler (Andrews *et al.*, 1988). Harding (1995) reviews how problems of microcrystal diffraction can be overcome. A large number of synthetic chemist users with small crystals are queueing up to use new facilities like station 9.8 at Daresbury, which came on-line in 1996 (Cernik *et al.*, 1997). Likewise, unstable compounds/crystals can benefit from the high intensity since much more rapid data collection is feasible with SR. *In situ* energy-dispersive diffraction monitoring of chemical reactions in a reacting chamber is described by Clark *et al.* (1995). The combined use of X-ray diffraction and EXAFS (see below) to study catalytic reactions *via* a sample cell is described by Shannon *et al.* (1997). The study of charge density, including SR, is described in this Special Issue by Coppens (1998).

In the study of disordered samples, other SR techniques besides single-crystal diffraction are obviously used. EXAFS probes the local environment of a metal atom yielding a radial distance distribution of nearest neighbours. This method (Stern *et al.*, 1975) and its interpretation based on low-energy electron diffraction (LEED) theory (Lee & Pendry, 1975) is utilized to study many compounds and materials. A most interesting development is in the rapid acquisition of EXAFS data to follow chemical reactions in a reaction cell in real time. Two approaches are possible: one is based on the dispersive monochromator ['dispersive EXAFS approach' (Matsushita & Phizackerley, 1981), and the other on quick scanning of a monochromator, the QEXAFS approach (Wong *et al.*, 1995)]. These are applicable to different time-sampling intervals, covering milliseconds to seconds, respectively, and with different applicabilities re sensitivity. For example, Hagelstein *et al.* (1997) have developed a beamline on an ESRF undulator for dispersive EXAFS. A time resolution of 0.1 ms is possible. Early experiments have included temperature-programmed desorption studies up to 630 K for 'PtY catalyst'. The reactant partial pressures were continuously monitored along with the EXAFS spectra at the Pt L_3 edge. It was found that the ammonia and water ligands desorb in two steps, at 380 and 540 K. Overall, the process, covered an elapsed time of 30 min (Fig. 8).

In the study of partially ordered samples (*e.g.* of fibres), a great deal of diffraction work has gone on within the fields of materials including polymer stretching, important for industrial applications (*e.g.* see Bras & Ryan, 1996; Hughes *et al.*, 1996). Studies of biological fibres, especially muscle, have already been referred to earlier in terms of their key phase in the history of SR and diffraction studies. See also Stuhrmann (1981) and Koch & Bordas (1983). I will highlight here a very recent example involving 2D order of a
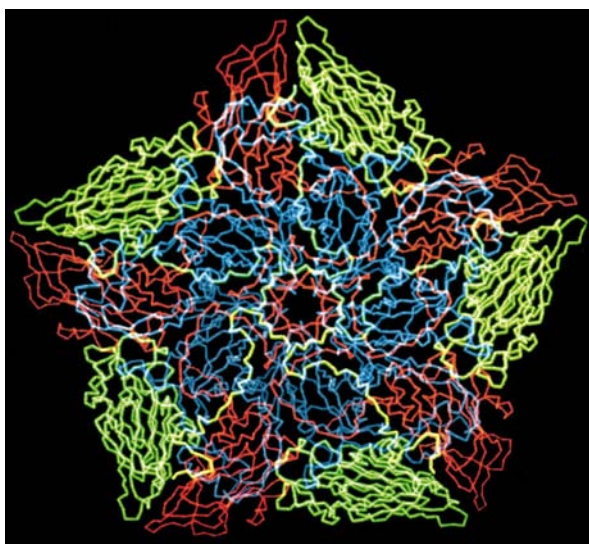
Fig. 11. A view of rhinovirus as determined from SR data measured at CHESS (based on Rossmann *et al.*, 1985). From the *Cornell Engineering Quarterly*, Spring 1986 issue, with permission.

Fig. 12. F_1 ATPase, one of the largest nonsymmetrical protein crystal structure complexes, solved using SR data recorded at Daresbury (see Figs. 3 and 5). The scale bar is 20 Å long. Reprinted with permission from *Nature* (Abrahams *et al.*, 1994). Copyright (1994) MacMillan Magazines Ltd.

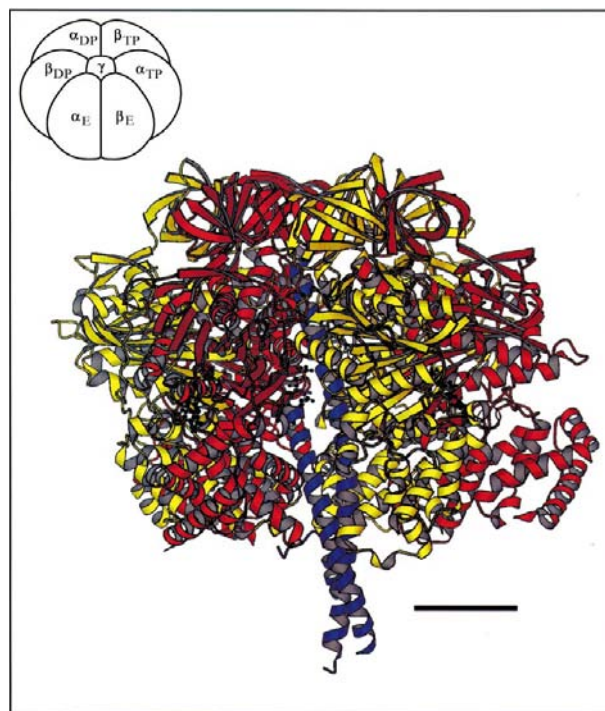different kind, namely molecules sitting on the surface of a solution. Grazing-incidence diffraction (GID) using SR is used to study surface monolayers. For example, Rapaport *et al.* (1997) undertook GID experiments on the liquid surface diffractometer (Als-Nielsen *et al.*, 1994) at the BW1 undulator in HASYLAB, Hamburg; the incident SR X-ray beam at grazing incidence illuminated an area of the surface of 5–50 mm. In a study (Fig. 9) of a valinomycin–Ba(ClO₄)₂ complex, the 2D rectangular unit cell was found to be essentially the same as the *ab* plane of the 3D X-ray crystal structure. As well as illustrating this technique, and its application, it is an interesting link between 2D and 3D molecular organization. Clearly, GID is a technique with a range of possible applications in terms of molecular 2D situations including industrial applications (sensors utilizing ionophores and so on) but also to characterize membranes. For membranes, a complementary approach is simultaneous small- and wide-angle X-ray scattering (see for an example Pressl *et al.*, 1997).

The interface between chemistry, biochemistry and molecular biology is a major growth area. In crystallography, the use of high SR intensity, cryo freezing of a protein crystal to largely overcome sample radiation damage and sensitive automatic area detectors (CCDs and/or image plates) are allowing a growing number of protein crystal structure studies at atomic resolution (Dauter *et al.*, 1997). The 'X-ray data-to-parameter' ratio can even be favourable enough for single and double bonds in carboxyl side chains, for example, to be resolved [Fig. 10 (Deacon *et al.*, 1997)]. Along with H atoms, this shows the details then to directly see the reactive proton. This approach complements H/D-exchange neutron diffraction studies. Neutron studies have recently expanded in scope by employing Laue geometry in a synergistic development with SR Laue diffraction (Helliwell, 1997*a,b*; Habash *et al.*, 1997). The scope and accuracy of protein crystal structures has been transformed. Time-resolved SR Laue diffraction of light-sensitive proteins, such as COMb is feasible with subnanosecond time resolution in pump probe reversible experiments [Srajer *et al.* (1996); and the article by Moffat (1998) in this Special Issue], are giving direct structural changes as a function of time. Enzyme, irreversible, processes are being studied directly by time-resolved methods *via* a variety of reaction initiation methods including *e.g.* pH jump, substrate diffusion and light flash of caged compounds pre-equilibrated in the crystal. Flash freezing is used to trap molecular structures at optimal times in a reaction determined either by microspectrophotometery or repeated Laue 'flash photography'. For overviews, see the books edited by Cruickshank *et al.* (1992) and Helliwell & Rentzepis (1997). These examples then lead us into applications of SR in biology. For an extensive survey of SR in chemistry, biochemistry and biology, see Mandelkow (1989).

6.3. *Applications in biology and medicine*

Multimacromolecular complexes such as viruses [Rossmann *et al.* (1985) and Fig. 11 (Acharya *et al.*, 1989), Liddington *et al.* (1991)] and the light harvesting complex (McDermott *et al.*, 1995), are very firmly recognizable as biological entities, whose crystal structure determinations relied on SR. These single-crystal structure determinations involved extremely large unit cells and are now tractable in spite of very weak sample scattering strength, and the crystals are often of unprecedented sensitivity to radiation (hundreds of, even a thousand, crystals have been used to constitute a single virus crystal data set). Cryocrystallography radiation protection is now used extensively in crystallographic data collection on whole ribosome crystals (Hope *et al.*, 1989); SR is essential for this structure determination (Yonath *et al.*, 1998, in this Special Issue). A major surge in results has come from ESRF where X-ray undulator radiation, of incredible intensity and collimation, in a number of beamlines (Miller, 1994; Branden, 1994) has been harnessed to yield atomic level crystal structures of the 780 Å diameter bluetongue virus (Grimes *et al.*, 1997; ESRF, 1997), and the nucleosome core particle (Luger *et al.*, 1997). A very large multiprotein complex solved using data from the Daresbury SRS wiggler is the F₁ ATPase structure (Fig. 12) for which a share in the Nobel Prize for Chemistry in 1997 was awarded to John Walker in Cambridge. The structure (Abrahams *et al.*, 1994; Abrahams & Leslie, 1996) and the amino-acid sequence data, along with fluorescence microscopy, show how biochemical energy is harnessed to drive the proton pump across biological membranes, thus corroborating hypotheses on this process over many years.

Rapid protein structure determination *via* the MAD method and improved heavy-atom isomorphous replacement data with wavelength-optimized anomalous scattering are removing a major bottleneck in protein crystallography, that of phase determination. There arises the possibility of considering whole genome-level protein structure determination (Chayen & Helliwell, 1998) of immense potential for understanding and controlling disease including rational drug design (*e.g.* see Ealick *et al.*, 1990; Bugg *et al.*, 1993).

7. Concluding remarks

SR and crystallography are now intricately intertwined in their scientific futures and in facilities provision. Crystallography is the largest anticipated user of SR in the UK, for example, as we approach the new millennium comprising more than 70% of the principal investigators in a recent survey (Suller, 1994). The history of SR X-ray provision started from high-energy physics at the outset and now has a vigorous future as exemplified by the planning of upgraded national X-ray sources (see *e.g.* Table 1). The prospects are bright in

terms of contributions to basic scientific research as well as applications in industry and medicine that SR and crystallography will make in the future.

References

Abrahams, J. P. & Leslie, A. G. W. (1996). *Acta Cryst.* D**52**, 30–42.

Abrahams, J. P., Leslie, A. G. W., Lutter, R. & Walker, J. E. (1994). *Nature (London)*, **370**, 621–628.

Acharya, R., Fry, E., Stuart, D., Fox, G., Rowlands, D. & Brown, F. (1989). *Nature (London)*, **337**, 709–716.

Akahama, Y., Kawamura, H., Hausermann, D., Hanfland, M. & Shimomura, O. (1995). *Phys. Rev. Lett.* **74**, 4690–4693.

Allinson, N. M. (1994). *J. Synchrotron Rad.* **1**, 54–62.

Als-Nielsen, J., Jacquemain, D., Kjaer, K., Leveiller, F., Lahav, M. & Leiserowitz, L. (1994). *Phys. Rep.* **246**, 251–313.

Amemiya, Y. (1997). *Methods Enzymol.* **276**, 233–243.

Andrews, S. J., Papiz, M. Z., McMeeking, R., Blake, A. J., Lowe, B. M., Franklin, K. R., Helliwell, J. R. & Harding, M. M. (1988). *Acta Cryst.* B**44**, 73–77.

Arndt, U. W., Greenhough, T. J., Helliwell, J. R., Howard, J. A. K., Rule, S. A. & Thompson, A. W. (1982). *Nature (London)*, **298**, 835–838.

Bergevin, F. de & Brunel, M. (1972). *Phys. Lett.* **39**A, 141–142.

Beuville, E., Beche, J. F., Cork, C., Douence, V., Earnest, T., Millaud, J., Nygren, D., Padmore, H., Turko, B., Zizka, G., Datte, P. & Xuong, N. H. (1997). *Nucl. Instrum. Methods*, **395**, 429–434.

Bilderback, D. H. (1986). *Nucl. Instrum. Methods*, **246**, 434–436.

Blewett, J. P. (1946). *Phys. Rev.* **69**, 87–95.

Bordas, J., Glazer, A. M. & Munro, I. H. (1976). *Nature (London)*, **262**, 541–545.

Bourenkov, G. P., Popov, A. N. & Bartunik, H. D. (1996). *Acta Cryst.* A**52**, 797–811.

Brammer, R., Helliwell, J. R., Lamb, W., Liljas, A., Moore, P. R., Thompson, A. W. & Rathbone, K. (1988). *Nucl. Instrum. Methods*, A**271**, 678–687.

Branden, C. I. (1994). *Structure*, **2**, 5–6.

Bras, W. & Ryan, A. J. (1996). *J. Mol. Struct.* **383**, 309–314.

Brinkmann, R., Materlik, G., Rossbach, J., Schneider, J. R. & Wilk, B. H. (1997). *Nucl. Instrum. Methods*, **393**, 86–92.

Brunel, M., Patrat, G., de Bergevin, F., Rousseaux, F. & Lemonnier, M. (1983). *Acta Cryst.* A**39**, 84–88.

Bugg, C. E., Carson, W. M. & Montgomery, J. A. (1993). *Sci. Am.* No. 12, pp. 60–66.

Buras, B. & Gerward, L. (1975). *Acta Cryst.* A**31**, 372–374.

Caspar, D. L. D., Clarage, J., Salunke, D. M. & Clarage, M. (1988). *Nature (London)*, **352**, 659–662.

Cauchois, Y., Bonnelle, C. & Missoui, G. (1963). *C. R. Acad. Sci.* **257**, 409–412.

Cernik, R. J., Clegg, W., Catlow, C. R. A., Bushnell-Wye, G., Flaherty, J. V., Greaves, G. N., Burrows, I., Taylor, D. J., Teat, S. J. & Hamichi, M. (1997). *J. Synchrotron Rad.* **4**, 279–286.

Chang, S.-L. (1998). *Acta Cryst.* A**54**, 886–894.

Charpak, G. (1970). *Annu. Rev. Nucl. Sci.* **20**, 195.

Chayen, N. E., Boggon, T. J., Cassetta, A., Deacon, A., Gleichmann, T., Habash, J., Harrop, S. J., Helliwell, J. R., Nieh, Y. P., Peterson, M. R., Raftery, J., Snell, E. H., Hädener, A., Niemann, A. C., Siddons, D. P., Stojanoff, V., Thompson, A. W., Ursby, T. & Wulff, M. (1996). *Q. Rev. Biophys.* **29**, 227–278.

Chayen, N. E. & Helliwell, J. R. (1998). *Phys. World*, No. 5, pp. 43–48.

Clark, S. M., Nield, A., Rathbone, T., Flaherty, J., Tang, C. C., Evans, J. S. O., Francis, R. J. & O'Hare, D. (1995). *Nucl. Instrum. Methods*, **97**, 98–101.

Coppens, P. (1992). *Synchrotron Radiation Crystallography*. New York: Academic Press.

Coppens, P. (1998). *Acta Cryst.* A**54**, 779–788.

Cruickshank, D. W. J., Helliwell, J. R. & Johnson, L. N. (1992). Editors. *Time-Resolved Macromolecular Crystallography*. Oxford University Press/The Royal Society.

Cruickshank, D. W. J., Helliwell, J. R. & Moffat, K. (1987). *Acta Cryst.* A**43**, 656–674.

Cruickshank, D. W. J., Helliwell, J. R. & Moffat, K. (1991). *Acta Cryst.* A**47**, 352–373.

Dauter, Z., Lamzin, V. S. & Wilson, K. S. (1997). *Curr. Opin. Struct. Biol.* **7**, 681–688.

Deacon, A., Gleichmann, T., Kalb (Gilboa), A. J., Price, H., Raftery, J., Bradbrook, G., Yariv, J. & Helliwell, J. R. (1997). *J. Chem. Soc. Faraday Trans.* **93**, 4305–4312.

Doniach, S., Hodgson, K. O., Lindau, I., Pianetta, P. & Winick, H. (1997). *J. Synchrotron Rad.* **4**, 380–395.

Doucet, J. & Benoit, J. P. (1987). *Nature (London)*, **325**, 643–646.

Ealick, S. E., Rule, S. A., Carter, D. C., Greenhough, T. J., Babu, Y. S., Cook, W. J., Habash, J., Helliwell, J. R., Stoeckler, J. D., Parks, R. E. Jr, Chen, S. & Bugg, C. E. (1990). *J. Biol. Chem.* **265**, 1812–1820.

Eikenberry, E. F., Barna, S. L., Tate, M. W., Rossi, G., Wixted, R. L., Sellin, P. J. & Gruner, S. M. (1998). *J. Synchrotron Rad.* **5**, 252–255.

Einspahr, H., Suguna, K., Suddath, F. L., Ellis, G., Helliwell, J. R. & Papiz, M. Z. (1985). *Acta Cryst.*, B**41**, 336–341.

Elder, F. R., Gurewitsch, A. M., Langmuir, R. V. & Pollack, H. C. (1947). *Phys. Rev.* **71**, 829.

Elder, F. R., Langmuir, R. V. & Pollack, H. C. (1948). *Phys. Rev.* **74**, 52–56.

Elleaume, P. (1989). *Synchrotron Rad. News*, **1**(4), 18–23.

Elleaume, P. (1998). *J. Synchrotron Rad.* **5**, 1–5.

Elleaume, P., Chavanne, J. & Van Vaerenbergh, P. (1998). *J. Synchrotron Rad.* **5**, 196–201.

ESRF (1987). ESRF Foundation Phase Report, Grenoble, France.

ESRF (1997). *ESRF Highlights 1996/1997*, Grenoble, France.

Fourme, R. (1997). *Nucl. Instrum. Methods*, A**392**, 1–11.

Gibbs, D., Harshmann, D. R., Isaacs, E. D., McWhan, D. B., Mills, D. & Vettier, C. (1988). *Phys. Rev. Lett.* **61**, 1241–1244.

Glover, I. D., Harris, G. W., Helliwell, J. R. & Moss, D. S. (1991). *Acta Cryst.* B**47**, 960–968.

Grimes, J. M., Jakana, J., Ghosh, M., Basak, A. K., Roy, P., Chiu, W., Stuart, D. I. & Prasad, B. V. V. (1997). *Structure*, **5**, 885–893.

Guss, J. M., Merritt, E. A., Phizackerley, R. P., Hedman, B., Murata, M., Hodgson, K. O. & Freeman, H. C. (1988). *Science*, **241**, 806–811.

Habash, J., Raftery, J., Weisgerber, S., Cassetta, A., Lehmann, M. S., Hoghoj, P., Wilkinson, C., Campbell, J. W. & Helliwell, J. R. (1997). *J. Chem. Soc. Faraday. Trans.* **93**, 4313–4317.

Hagelstein, M., San Miguel, A., Fontaine, A. & Goulon, J. (1997). *J. Phys. (Paris) IV*, **7**, No. C2, Part 1, pp. 303–308.

Hajdu, J., Acharya, K. R., Stuart, D. I., McLaughlin, P. J., Barford, D., Oikonomakos, N. G., Klein, H. & Johnson, L. N. (1987*a*). *EMBO J.* **6**, 539–546.

Hajdu, J., Machin, P. A., Campbell, J. W., Greenhough, T. J., Clifton, I. J., Zurek, S., Gover, S., Johnson, L. N. & Elder, M. (1987*b*). *Nature (London)*, **329**, 178–181.

Hall, G. (1995). *Q. Rev. Biophys.* **28**, 1–32.

Harding, M. M. (1995). *Acta Cryst.*. B**51**, 432–446.

Harmsen, A., Leberman, R. & Schultz, G. E. (1976). *J. Mol. Biol.* **104**, 311–314.

Hart, M. & Berman, L. (1998). *Acta Cryst.* A**54**, 850–858.

Haslegrove, J. C., Faruqi, A. R., Huxley, H. E. & Arndt, U. W. (1977). *J. Phys. E*, **10**, 1035–1044.

Helliwell, J. R. (1984). *Rep. Prog. Phys.* **47**, 1403–1497.

Helliwell, J. R. (1985). *J. Mol. Struct.* **130**, 63–91.

Helliwell, J. R. (1992). *Macromolecular Crystallography with Synchrotron Radiation*. Cambridge University Press.

Helliwell, J. R. (1997*a*). *Methods Enzymol.* **276**A, 203–217.

Helliwell, J. R. (1997*b*). *Nature Struct. Biol.* **4**, 874–876.

Helliwell, J. R., Habash, J., Cruickshank, D. W. J., Harding, M. M., Greenhough, T. J., Campbell, J. W., Clifton, I. J., Elder, M., Machin, P. A., Papiz, M. Z. & Zurek, S. (1989). *J. Appl. Cryst.* **22**, 483–497.

Helliwell, J. R., Papiz, M. Z., Glover, I. D., Habash, J., Thompson, A. W., Moore, P. R., Harris, N., Croft, D. & Pantos E. (1986). *Nucl. Instrum. Methods*, A**246**, 617–623.

Helliwell, J. R. & Rentzepis, P. M. (1997). Editors. *Time-Resolved Diffraction*. Oxford University Press.

Hendrickson, W. A., Pahler, A., Smith, J. L., Satow, Y., Merritt, E. A. & Phizackerley, R. P. (1989). *Proc. Natl Acad. Sci. USA*, **86**, 2190–2194.

Herzenberg, A. & Lau, H. S. M. (1967). *Acta Cryst.* **22**, 24–28.

Hope, H., Frolow, F., von Bohlen, K., Makowski, I., Kratky, C., Halfon, Y., Dauz, H., Webster, P., Bartels, K. S., Wittmann, H. G. & Yonath, A. (1989). *Acta Cryst.* B**45**, 190–199.

Hoppe, H. & Jakubowski, V. (1975). *Anomalous Scattering*, edited by S. Ramaseshan & S. C. Abrahams, pp. 437–461. Copenhagen, Munksgaard.

Hughes, D. J., Mahendrasingam, A., Heeley, E. L., Oatway, W. B., Martin, C., Towns-Andrew, E. & Fuller, W. (1996). *J. Synchrotron Rad.* **3**, 84–90.

Huxley, H. E. & Holmes, K. C. (1997). *J. Synchrotron Rad.* **4**, 366–379.

Iwanenko, D. & Pomeranchuk, I. (1944). *Phys. Rev.* **65**, 343.

Kahn, R., Fourme, R., Bosshard, R., Chiadmi, M., Risler, J. L., Dideberg, O. & Wery, J. P. (1985). *FEBS Lett.* **170**, 133–137.

Karle, J. (1967). *Appl. Opt.* **6**, 2132–2135.

Karle, J. (1980). *Int. J. Quantum Chem. Symp.* **7**, 357–367.

Koch, M. H. J. & Bordas, J. (1983). *Nucl. Instrum. Methods*, **208**, 461–469.

Korszun, Z. R. (1987). *J. Mol. Biol.* **196**, 413–419.

Lee, P. A. & Pendry, J. (1975). *Phys. Rev. B*, **11**, 2795–2811.

Lewis, R. A. (1994). *J. Synchrotron Rad.* **1**, 43–53.

Liddington, R. C., Yan, Y., Moulai, J., Sahli, R., Benjamin, T. L. & Harrison, S. C. (1991). *Nature (London)*, **354**, 278–284.

Lippert, M., Brucknel, T., Kohler, T. & Schneider, J. R. (1994). *Europhys. Lett.* **27**, 537–541.

Lovesey, S. W. & Collins, S. P. (1996). *X-ray Scattering and Absorption by Magnetic Materials*. Oxford University Press.

Luger, K., Mader, A. W., Richmond, R. K., Sargent, D. F. & Richmond, T. J. (1997). *Nature (London)*, **389**, 251–260.

McDermott, G., Prince, S. M., Freer, A. A., Hawthornthwaite-Lawless, A. M., Papiz, M. Z., Cogdell, R. J. & Isaacs, N. W. (1995). *Nature (London)*, **374**, 517–521.

McWhan, D. B., Vettier, C., Isaacs, E. D., Ice, G. E., Siddons, D. P., Hastings, J. B., Peters, C. & Vogt, O. (1990). *Phys. Rev. B*, **42**, 6007–6017.

Mandelkow, E. (1989). Editor. *Synchrotron Radiation in Chemistry and Biology. Topics in Current Chemistry*, Vol. 151, pp. 1–229. Berlin: Springer Verlag.

Matsushita, T. & Phizackerley, R. P. (1981). *Jpn. J. Appl. Phys.* **20**, 2223–2228.

Miller, A. (1994). In *SR in Biophysics*, edited by S. S. Hasnain. Chichester: Ellis Hall.

Miyahara, J., Takahashi, K., Amemiya, Y., Kamiya, N. & Satow, Y. (1986). *Nucl. Instrum. Methods*, A**246**, 572–578.

Moffat, K. (1998). *Acta Cryst.* A**54**, 905–913.

Moffat, K., Szebenyi, D. & Bilderback, D. H. (1984). *Science*, **223**, 1423–1425.

Mossbauer, R. L. (1975). *Anomalous Scattering*, edited by S. Ramaseshan & S. C. Abrahams, pp. 463–483. Copenhagen: Munksgaard.

Mukherjee, A. K., Helliwell, J. R. & Main, P. (1989). *Acta Cryst.* A**45**, 715–718.

Munro, I. H. (1997). *J. Synchrotron Rad.* **4**, 344–358.

Neutze, R. & Hajdu, J. (1997). *Proc. Natl Acad. Sci. USA*, **94**, 5651–5655.

Okaya, Y. & Pepinsky, R. (1956). *Phys. Rev.* **103**, 1645–1647.

Parak, F., Mossbauer, R. L., Biebl, U., Formanek, H. & Hoppe, W. (1971). *Z. Phys.* **244**, 456–467.

Parratt, L. G. (1959). *Rev. Sci. Instrum.* **30**, 297–299.

Peterson, M. R., Harrop, S. J., McSweeney, S. M., Leonard, G. A., Thompson, A. W., Hunter, W. N. & Helliwell, J. R. (1996). *J. Synchrotron Rad.* **3**, 24–34.

Phillips, J. C., Templeton, L. K., Templeton, D. H. & Hodgson, K. O. (1978). *Science*, **201**, 257–259.

Phillips, J. C., Wlodawer, A., Yevitz, M. M. & Hodgson, K. O. (1976). *Proc. Natl Acad. Sci. USA*, **73**, 128–132.

Phizackerley, R. P., Rek, Z. U., Conradson, S. D., Hodgson, K. O. & Matsushita, T. (1982). *Nucl. Instrum. Methods*, **195**, 405.

Pressl, K., Jorgensen, K. & Laggner, P. (1997). *Biochim. Biophys. Acta Biomembr.* **1325**, 1–7.

Rapaport, H., Kuzmenko, I., Kjaer, K., Howes, P., Bouwman, W., Als-Nielsen, J., Leiserowitz, L. & Lahav, M. (1997). *J. Am. Chem. Soc.* **119**, 11211–11216.

Rosenbaum, G., Holmes, K. C. & Witz, J. (1971). *Nature (London)*, **230**, 434–437.

Rossmann, M. G., Arnold, E., Erickson, J. W., Frankenberger, E. A., Griffith, J. P., Hecht, H.-J., Johnson, J. E., Kamer, G., Luo, M., Mosser, A. G., Rueckert, R. R., Sherry, B. & Vriend, G. (1985). *Nature (London)*, **317**, 145–153.

Rossmann, M. G. & Erickson, J. W. (1983). *J. Appl. Cryst.* **16**, 629–636.

Rüffer, R. (1992). *Synchrotron Rad. News*, No. 5, pp. 25–29.

Sakabe, N., Ikemizu, S., Sakabe, K., Higashi, T., Nagakawa, A. & Watanabe, N. (1995). *Rev. Sci. Instrum.* **66**, 1276–1281.

Schwinger, J. (1949). *Phys. Rev.* **75**, 1912–1925.

Shannon, I. J., Maschmeyer, T., Sankar, G., Thomas, J. M., Oldroyd, R. D., Sheehy, M., Madill, D., Waller, A. M. & Townsend, R. P. (1997). *Catal. Lett.* **44**, 23–27.

Skuratovskii, I. Ya., Kapitonova, K. A. & Volkova, L. I. (1978). *J. Appl. Cryst.* **11**, 238–242.

Srajer, V., Teng, T. Y., Ursby, T., Pradervand, C., Ren, Z., Adachi, S., Schildkamp, W., Bourgeois, D., Wulff, M. & Moffat, K. (1996). *Science*, **274**, 1726–1729.

Stern, E. A., Sayers, D. E. & Lytle, F. W. (1975). *Phys. Rev. B*, **11**, 4836–4846.

Stuhrmann, H. B. (1981). *Q. Rev. Biophys.* **14**, 433–462.

Suller, V. (1994). *J. Synchrotron Rad.* **1**, 5–11.

Suller, V. (1998). Personal communication.

Tate, M. W., Eikenberry, E. F., Barna, S. L., Wall, M. E., Lowrance, J. L. & Gruner, S. M. (1995). *J. Appl. Cryst.* **28**, 196–205.

Templeton, D. H. & Templeton, L. K. (1985). *Acta Cryst.* A**41**, 365–371.

Usha, R., Johnson, J. E., Moras, D., Thierry, J. C., Fourme, R. & Kahn, R. (1984). *J. Appl. Cryst.* **17**, 147–153.

Webb, N. G., Samson, S., Stroud, R. M., Gamble, R. C. & Baldeschwieler, J. D. (1977). *J. Appl. Cryst.* **10**, 104–110.

Westbrook, E. M. & Naday, I. (1997). *Methods Enzymol.* **276**, 244–268.

Willis, B. T. M. (1998). *Acta Cryst.* A**54**, 914–921.

Winick, H. (1995). *J. Electron Spectrosc. Relat. Phenom.* **75**, 1–8.

Wong, J., Froba, M. & Frahm, R. (1995). *Physica (Utrecht)*, B**209**, 249–250.

Yonath, A., Harms, J., Hansen, H. A. S., Bashan, A., Schlünzen, F., Levin, I., Koelln, I., Tocilj, A., Agmon, I., Peretz, M., Bartels, H., Bennett, W. S., Krumbholz, S., Janell, D., Weinstein, S., Auerbach, T., Avila, H., Piolleti, M., Morlang, S. & Franceschi, F. (1998). *Acta Cryst.* A**54**, 945–955.