

# Application of synchrotron radiation in X-ray diffraction studies of crystal structures

Zbigniew Dauter

*European Molecular Biology Laboratory, Outstation Hamburg, c/o DESY, Notkestrasse 85, 22603 Hamburg, Germany*

Received 24 February 1995; accepted in final form 15 June 1995

## Abstract

The use of synchrotron radiation in studies of crystal structures by X-ray diffraction is reviewed. The beam lines and detectors used for diffraction experiments are described and the special characteristics of synchrotron X-ray radiation discussed.

## Introduction

This paper presents an overview of the various applications of X-ray radiation generated at synchrotron storage rings in the investigation of crystal structures. More detailed information can be found in comprehensive books and reviews, for example [1,2]. The importance of synchrotron sources is widely acknowledged in macromolecular crystallography [3] and a literature search showed that between January 1992 and June 1993 more than 110 structures of macromolecules based on synchrotron data were published [4]. Synchrotron radiation also has some important applications in diffraction studies of smaller structures [5]. Several new synchrotrons are being built in different countries and the availability to the international crystallographic community of beam lines dedicated to diffraction studies by X-rays will certainly increase in the near future.

Synchrotrons are machines which accelerate charged particles, such as electrons or positrons, to relativistic speed, giving them energy up to several GeV. The first particle accelerators were

constructed by high-energy physicists investigating subatomic phenomena observed when particles with very high relative energy collide. For these experiments, the electromagnetic radiation generated by orbiting particles was an unwanted side effect, causing a loss of energy by the particles. In the early sixties, it was realised that synchrotron radiation had properties which could be used in many fields of science, and since then several rings have been exclusively dedicated to producing synchrotron radiation.

Synchrotrons are used only to accelerate particles to the desired energy and to inject them into so-called storage rings where they are kept at constant speed (and energy). Synchrotron and storage rings are evacuated to a pressure of about  $10^{-12}$  torr to minimise particle loss by collisions with residual gas atoms. The ring current usually reaches several tens or hundreds of milliamperes. The particles orbit in a closed, more or less circular trajectory, consisting of straight sections and bending sectors controlled by strong bending electromagnets, which can be superconducting.

The trajectory of these particles is bent under the

influence of the strong magnetic field and the resulting angular acceleration causes them to emit strong electromagnetic radiation in a broad energy range, from microwaves (wavelength of the order of 1 m) through infrared, visible, ultraviolet light, to hard X-rays (less than 0.5 Å). This makes synchrotron radiation applicable in many fields of spectroscopic techniques and the high-energy edge of the spectrum, 0.3–2 Å, can be used for X-ray diffraction experiments on crystalline samples.

At present, the following synchrotrons provide radiation for the worldwide user community:

Synchrotron	Location
ESRF	Grenoble, France
DESY	Hamburg, Germany
LURE	Orsay, France
SRS	Daresbury, UK
ELETTRA	Trieste, Italy
VEPP	Novosibirsk, Russia
PHOTON FACTORY	Tsukuba, Japan
NSLS	Brookhaven, USA
CHSS	Ithaca, USA
SSRL	Stanford, USA

Several more synchrotrons are being built, e.g. in the United States, Japan, Brazil, South Korea and Taiwan, which will be available in the near future.

## 2. Synchrotron beam lines

The radiation generated at the synchrotron (or storage ring) cannot be used directly for experiments as it covers a very broad range of wavelengths and is not collimated, and, because of its enormous intensity, constitutes a very serious hazard to experimentalists. At every synchrotron site several beam lines are constructed. A typical crystallographic beam line must contain a source, either a bending magnet or an insertion device, optical elements such as a monochromator and focusing mirrors, several pairs of vertical and horizontal collimating slits and, at the end, an experimental table with final collimator, sample and detector. A beam line must, like the ring, be evacuated and usually ends with a beryllium window. An example of a synchrotron wiggler beam line BW7B of EMBL [6] at DORIS storage ring, DESY, Hamburg, is shown schematically in Fig. 1.

A bending magnet is an instrument which, as its name suggests, bends the trajectory of the particles, serving the dual purpose of making them orbit in a closed circuit and simultaneously being a source of radiation. The source has dimensions of up to 2 mm vertically and 5 mm horizontally. The radiation from the source forms a horizontal fan several milliradians wide. It is possible to divide one fan into two or more separate beam lines.

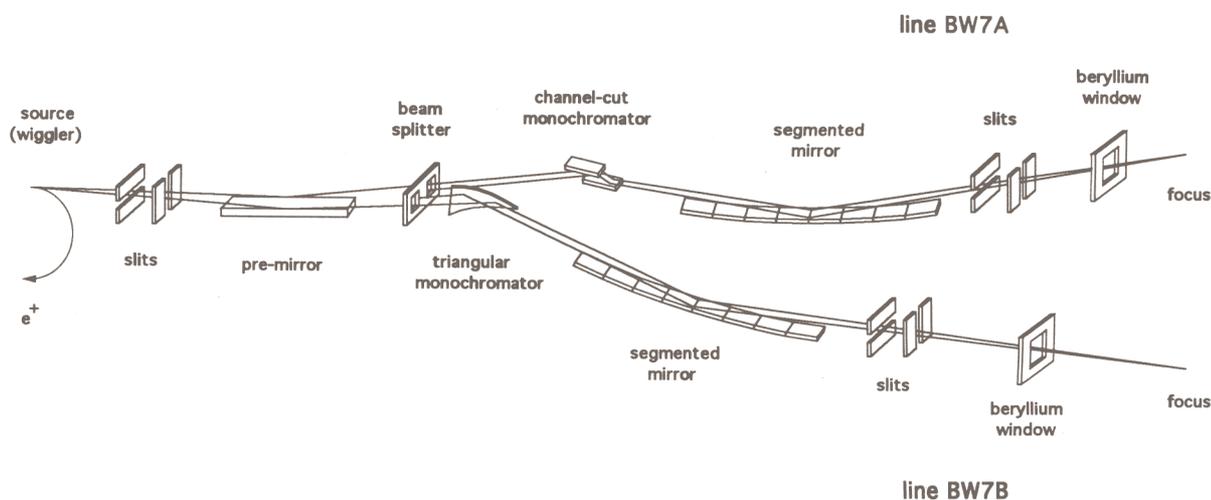


Fig. 1. Scheme of EMBL wiggler beam line BW7 in HASYLAB at DESY, Hamburg.

Between the magnets the particles move along straight sections, where it is possible to install so-called insertion devices, either wigglers or undulators. A wiggler is a magnet with many pairs of poles, so that particles are influenced by several “kicks”, thus emitting radiation more than an order of magnitude stronger than from a bending magnet. An undulator is in principle similar, but tuned so that radiation emitted from consecutive magnetic poles interferes positively along its line of propagation. It is characterised by strong spikes at particular wavelengths, which are at least an order of magnitude more intense than wiggler radiation.

For Laue diffraction, a stationary crystal is irradiated by the white beam with a wide range of wavelengths and there is no need to use a monochromator, perhaps only some means of eliminating very long wavelengths, such as a block of aluminium. For conventional single-wavelength diffraction experiments, a monochromator is required. There are two principal types of X-ray monochromators used in synchrotron beam lines.

The simpler type consists of a single crystal, usually made of silicon or germanium, cut parallel to the direction of strongly diffracting planes, e.g. (111) or (220), in the form of a long triangle, which can be bent to provide focusing in the bending plane (usually horizontal). The other type is a doubly diffracting channel-cut single crystal or parallel double-crystal monochromator. The first type provides a wider wavelength bandpass and hence more intensity; the latter gives a narrower bandpass, which is more appropriate for precise anomalous diffraction measurements. Changing the wavelength requires the monochromator to be rotated to utilise a different Bragg angle. In the first case the whole beam line beyond the monochromator must be moved, whereas for the doubly diffracting monochromator, change of wavelength does not require any significant movement of the remaining optical elements and tuning the wavelength can be achieved quickly and effectively.

Focusing of the beam is carried out by the reflecting mirror. There are various kinds of mirrors, usually made of glass, but they should be coated on the surface by a layer of heavy metal,

giving the smallest possible glancing angle and high reflectivity. The mirror can be in the form of one precisely shaped piece or it can consist of several prealigned elements. These have a toroidal shape to focus the beam in the horizontal plane and can be bent elliptically to provide focusing in the vertical plane. Depending on the ratio between source and focusing mirror and mirror-to-sample distances, the focus size can be diminished (so-called demagnification), thus providing additional enhancement of intensity. Sometimes no mirrors are used, e.g. undulators produce an X-ray beam of such small divergence that mirrors are not necessary. Additional mirrors can be used at the beginning of the beam line to cut out radiation of very long and very short wavelength.

The beam line elements are enclosed in a high vacuum and all their movements and alignments have to be executed by stepping motors controlled remotely through computers. Other important devices are beam shutters, which permit us to shut down and open the beam line when necessary. They usually consist of a block of metal and are controlled pneumatically.

After the X-rays leave the beam line through the beryllium window and enter the experimental hutch, they must be directed onto the sample through some additional collimating system. Its size should correspond to the size of the sample, usually 0.1–0.5 mm<sup>2</sup>. The beam in the hutch should be shielded to minimise the background radiation reaching the detector and interfering with the measured signal. The collimator may be equipped with ionisation chambers monitoring the beam intensity and used in the positional optimisation of the experimental set-up consisting of the collimator, sample holder and detector. The stepping motors moving these elements are controlled remotely by the computer. At the end of the collimator there is usually a small electromagnetically controlled shutter, which opens at the beginning and closes at the end of every exposure.

The single crystal mounted on the goniometer head must have the possibility of rotation around at least one axis or more if, for example, a  $\kappa$  goniostat is used.  $\chi$  goniostats are also used, although they require more free space around the sample. Protein samples are usually enclosed within a

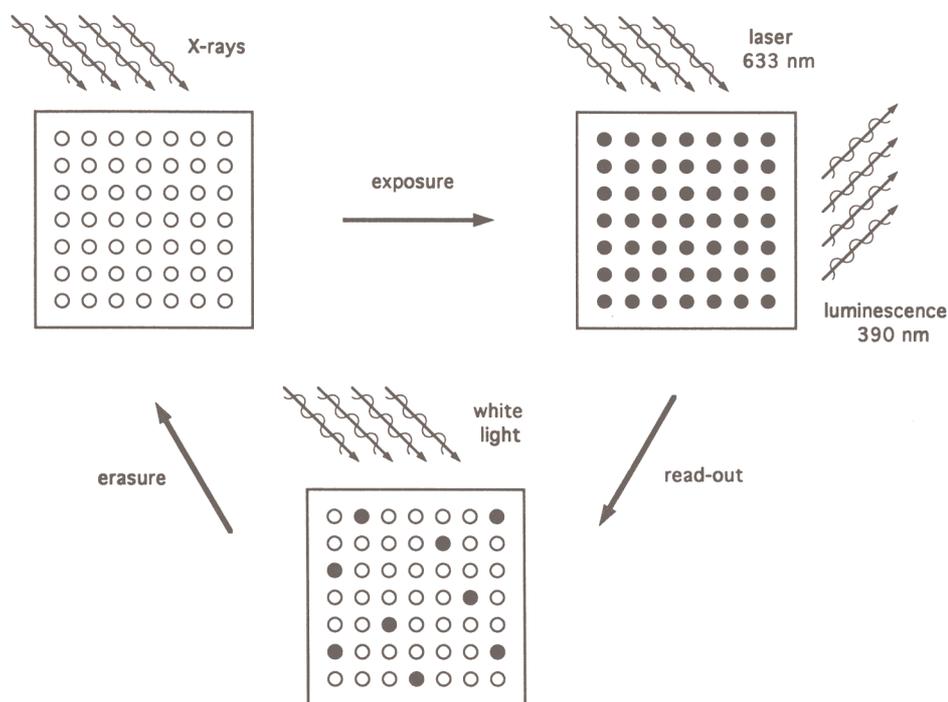


Fig. 2. A cycle of imaging plate operation, consisting of exposure to X-rays, read-out of photoluminescence caused by laser light, and erasure of remaining signal by white light.

sealed glass or quartz capillary with some mother liquor for room-temperature experiments, or kept within fibre loops in a drop of frozen solution. Small-structure crystals may be glued at the tip of a glass rod, as is standard in conventional diffractometry. Polycrystalline samples can be placed in glass capillaries. If the temperature of the experiment has to be controlled, this is usually achieved by a stream of gas (nitrogen) blown onto the sample. The alignment of the sample can be made easier with the use of a video camera viewing the crystal through a microscope.

### 3. Detectors

Four-circle single-counter diffractometers are used on some synchrotron beam lines, but they are practical only for rather small structures. For example, very accurate data showing anomalous dispersion of nitrogen atoms in boron nitride have been collected using a five-circle

diffractometer in HASYLAB at DESY in Hamburg [7]. The measurement of reflection intensities one by one requires a long time and, for crystals with large cell dimensions, where several thousands or, as for large proteins or viruses, hundreds of thousands of reflections have to be measured, the only practical solution is the use of two-dimensional area detectors coupled with the screenless rotation method for collecting X-ray data. Several types of area detectors are in use, each of which has certain advantages and disadvantages of synchrotron beam lines.

The classic two-dimensional detector is photographic film. It has one superior property—very high spatial resolution, better than  $25\ \mu\text{m}$ . Its other properties disqualify film from use in collecting X-ray data at synchrotron sites: limited dynamic range, low signal-to-noise ratio because of chemical fog (background), low sensitivity at short wavelengths and a lot of manual labour necessary for preparation, processing and subsequent optical scanning. Although a few years ago

film was the most widely used means of collecting data, it ceased to be used at synchrotron sites in favour of automatic detectors.

Detectors based on TV-cameras can be used, although, in spite of their high read-out speed, they have limited dynamic range. More popular, especially for the collection of very accurate data, are multi-wire proportional chamber devices, which are counting detectors. They provide high speed, but again are limited in dynamic range. As their read-out time is fast, TV and MWPC detectors are mostly used in fine slicing mode, where exposures are short and frequent.

A few years ago imaging-plate phosphors became the preferred type of detector to be used at synchrotron beam lines [8]. Fig. 2 shows the operating cycle of an imaging plate. An imaging plate stores the incident X-rays in the form of metastable excited europium ions which can subsequently be read-out by red laser light, causing the plate to emit blue light with intensity proportional to the amount of the integrated X-ray quanta. This light can be measured by a photomultiplier and information stored on the computer disk, pixel by pixel. Imaging plates have high dynamic range, highly linear response, good spatial resolution and may have large dimensions, but they are not very fast, requiring a few minutes after every exposure to read-out the image. Following the introduction of imaging-plate scanners in the late eighties [9,10], almost all macromolecular synchrotron beam lines are now equipped with them.

The latest kind of detectors used to collect X-ray data, especially at synchrotrons, are charge-coupled devices (CCDs) [11]. They are based on semiconductors able to integrate radiation incident on the active surface and to store the information within every pixel automatically on the computer disk. CCDs have similar properties to imaging plates but have faster read-out, which is advantageous at synchrotron sites.

The scattered X-ray intensity is measured by a two-dimensional area detector in every pixel within the active window and this information is stored, possibly after correcting for different response of different pixels. To obtain the intensities of diffracted reflections, it is necessary to integrate the total intensity within individual

reflection profiles, taking into account appropriate background and other corrections. Several programs performing this task are available. The integration algorithm differs slightly between fine-slicing and wide-oscillation approaches. For wide oscillations, when the rotation range is wider than the total divergence of the diffracted beam (convoluted with the mosaicity of the sample crystal), most reflections are fully recorded on a single image or their intensity is divided between two successive images (partially recorded). The intensity peaks are represented as two-dimensional profiles for integration. In the fine-slicing method, when every intensity peak is spread over several consecutive images, a three-dimensional profile can be built for integration.

All integrating algorithms involve initial indexing of the diffraction pattern and refinement of parameters, dependent on the crystal cell and orientation and on characteristics of the beam and detector, minimising the discrepancy between observed and predicted spot positions. Integration can be done off-line at the end of data collection (the usual case in wide-oscillation mode) or on-line after recording each image (practical in fine-slicing mode).

After integration, intensities from different images have to be scaled and symmetry-equivalent reflections merged to produce the unique data ready for further crystallographic calculations.

#### 4. Properties of synchrotron radiation

Synchrotron radiation has several advantageous properties in X-ray crystallography. The X-ray intensity is several orders of magnitude higher than that of sealed-tube or rotating-anode sources. It allows the collection of diffraction data on very small or very weakly diffracting crystals. This is especially important for viruses or large proteins, where the intense beam, coupled with efficient two-dimensional detectors, is crucial for obtaining measurable diffraction. Very strong radiation may damage the crystal, especially for large macromolecules through absorption of energy, which may lead to breaking of chemical bonds, formation of radicals and chemical reactions within the

sample. However, the strong beam seems to allow us to make more use of the crystal, as radiation damage is often kinetically controlled and requires time to affect the crystal. This problem can be almost completely alleviated by freezing the sample down to liquid nitrogen temperature. If done properly and rapidly, this does not lead to formation of ice because water within protein crystals freezes as glass. When so frozen, even very delicate protein crystals are practically “immortal” in the beam.

The high intensity of synchrotron radiation allows us to study very large structures, comprising hundreds of thousands of atoms, like those of light-harvesting photosystem I [12], virus capsids [13] or whole ribosomes [14].

A synchrotron X-ray beam can be highly collimated and focused on samples often smaller than 0.1 mm. Low divergence of the beam, coupled with often extremely small mosaicity of protein crystals, is advantageous because the signal-to-noise ratio is enhanced and it is possible to collect meaningful data from very small and weakly diffracting crystals. Fine collimation is important for very large cells, producing small but not overlapping reflection profiles on the detector. This is also important in powder crystallography, where a highly collimated and strong synchrotron beam allows one to record more significantly weak powder diffraction bands [15,16].

Another important property of synchrotron radiation is the possibility of tuning the wavelength of the desired value. This can be used to optimise the anomalous dispersion signal, if the sample contains metals or other elements having K or L absorption edges in the available wavelength range. A significant anomalous signal helps one to solve the phase problem in protein crystallography, by using either optimised anomalous scattering measurements or multi-wavelength anomalous diffraction (MAD).

Multiple isomorphous replacement requires preparation of isomorphous derivatives containing heavy atoms. Their contribution to the diffraction can be separated from that of the native protein and utilised to provide initial phases. If, in addition to the isomorphous effect, the anomalous scattering of the heavy metal is recorded as

differences between Bijvoet related intensities, this significantly enhances the reliability of the phases obtained. The presence of the anomalous dispersion signal makes it possible to differentiate between the two possible enantiomeric solutions of the structure.

MAD is based exclusively on the anomalous dispersion contribution of heavy atoms in the crystal [17,18]. It is applicable to metalloproteins or heavy atom derivatives without the need to collect native data separately. This avoids any problems with non-isomorphous derivatives, but requires very accurate intensity measurements. It is necessary to collect three or four data sets on the same crystal at different wavelengths in the vicinity of the metal absorption edge, with different contributions to the real  $\Delta f'$  and imaginary  $\Delta f''$  anomalous corrections. These can be utilised to obtain experimental estimates of the phases.

Using the non-monochromatised, so-called white beam opens up the possibility of collecting the whole data set very rapidly, in less than a fraction of a second by using the Laue method [19,20]. This gives the opportunity of tracing short-lived intermediate states along the enzymatic reaction path in the crystalline state [21]. In this technique, the crystal is stationary, but the wide wavelength range ensures that many reflections are excited simultaneously. The sample crystal must have very low mosaicity. The Laue method requires detectors with high spatial resolution to be able to resolve the very large number of spot profiles. The higher the symmetry of the crystal, the higher the proportion of the unique data which can be recorded on a single exposure. The Laue method has some intrinsic limitations. Low-order reflections give rise to multiplets on the detector, e.g. reflections with indices multiplied by  $n$  diffract at the same angle if the wavelength is  $n$  times shorter. It is possible to “unscramble” individual intensities within doublets, but not higher multiplets. Therefore Laue data are never complete, especially at low angle [22].

The particles in synchrotron rings are not evenly distributed but are grouped in bunches. Thus synchrotron radiation is not continuous, but consists of short, picosecond pulses with nanosecond gaps. This can be utilised in some

experiments to provide a means of extremely fast exposures [23]. This requires extremely fast beam shutters.

As the number of synchrotron sites and their availability through international scientific networks increase, it is clear that more users from the crystallographic community will benefit from the use of synchrotron radiation in crystallographic studies.

## References

- [1] P. Coppens, *Synchrotron Radiation Crystallography*, Academic Press, London, 1992.
- [2] J.R. Helliwell, *Macromolecular Crystallography with Synchrotron Radiation*, Cambridge, University Press, 1992.
- [3] J.R. Helliwell, *J. Mol. Struct.*, 130 (1985) 63.
- [4] S.E. Ealick and R.L. Walter, *Curr. Opinion Struct. Biol.*, 3 (1993) 725.
- [5] E. Pohl, A. Heine, G.M. Sheldrick, Z. Dauter, T.R. Schneider, K.S. Wilson and J. Kallen, *Acta Crystallogr.*, Sect. D, 51 (1995) 60.
- [6] R.G. van Silfhout and C. Hermes, *Rev. Sci. Instrum.*, 66 (1995) 1818.
- [7] K. Eichhorn, A. Kirfel, J. Grochowski and P. Serda, *Acta Crystallogr.*, Sect. B, 47 (1991) 843.
- [8] Y. Amemiya and J. Miyahara, *Nature*, 336 (1988) 89.
- [9] N. Sakabe, *Nucl. Instrum. Methods Phys. Res. A*, 303 (1991) 448.
- [10] Z. Dauter, H. Terry, H. Witzel and K.S. Wilson, *Acta Crystallogr.*, Sect. B, 46 (1990) 833.
- [11] S.M. Gruner and S.E. Ealick, *Structure*, 3 (1995) 13.
- [12] N. Krauss, W. Hinrichs, I. Witt, P. Fromme, W. Pritzkow, Z. Dauter, C. Betzel, K.S. Wilson, H.T. Witt and W. Saenger, *Nature*, 361 (1993) 326.
- [13] R. McKenna, D. Xia, P. Willingmann, L.L. Ilag, S. Krishnaswamy, M.G. Rossmann, N.H. Olson, T.S. Baker and N.L. Incardona, *Nature*, 355 (1992) 137.
- [14] A. Yonath, *Annu. Rev. Biophys. Biomol. Struct.*, 21 (1992) 77.
- [15] B.T.W. Willis, in D.W. Jones and A. Katrusiak (Eds.), *Correlations, Transformations and Interactions in Organic Crystal Chemistry*, I.U.Cr., Oxford, 1994, p. 11.
- [16] R.J. Nelmes and M.I. McMahon, *J. Synchrotron Rad.*, 1 (1994) 69.
- [17] W.A. Hendrickson, *Science*, 254 (1991) 51.
- [18] J.L. Smith, *Curr. Opinion Struct. Biol.*, 1 (1991) 1002.
- [19] J.R. Helliwell, J. Habash, D.W.J. Cruickshank, M.M. Harding, T.J. Greenhough, J.W. Campbell, I.J. Clifton, M. Elder, P.A. Machin, M.Z. Papiz and S. Zurek, *J. Appl. Crystallogr.*, 22 (1987) 483.
- [20] S. Gruner, *Science*, 238 (1987) 308.
- [21] I. Schlichting, S.C. Almo, G. Rapp, K.S. Wilson, K. Petratos, A. Lentfer, A. Wittinghofer, W. Kabsch, E.F. Pai, G.A. Petsko and R.S. Goody, *Nature*, 345 (1990) 309.
- [22] D.W.J. Cruickshank, J.R. Helliwell and K. Moffat, *Acta Crystallogr.*, Sect. A, 476 (1987) 656.
- [23] D.M.E. Szebenyi, D. Bilderback, A. LeGrand, K. Moffat, W. Schildkamp and T.Y. Teng, *Trans. Am. Crystallog. Assoc.*, 24 (1988) 167.