

## Impact of synchrotron radiation on macromolecular crystallography: a personal view

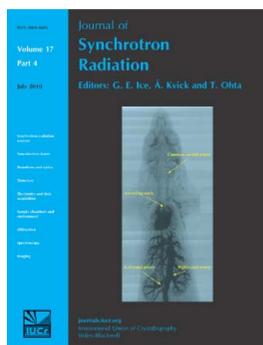
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# Impact of synchrotron radiation on macromolecular crystallography: a personal view

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The introduction of synchrotron radiation sources almost four decades ago has led to a revolutionary change in the way that diffraction data from macromolecular crystals are being collected. Here a brief history of the development of methodologies that took advantage of the availability of synchrotron sources are presented, and some personal experiences with the utilization of synchrotrons in the early days are recalled.

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## 1. Introduction

The birth of X-ray crystallography almost a century ago, and its first successful application a half century later to the elucidation of the structure of biological macromolecules, were the turning points in our ability to understand the molecules of life at the atomic level. Clearly, the success of this methodology has been critically dependent on the ability to generate sufficiently strong X-ray beams that would provide measurable diffraction images. The original sources of X-rays were variants of tubes in which electromagnetic radiation was produced by bombardment of anodes (made of metals such as copper or molybdenum) with electrons emitted by thermionic cathode (a hot wire) and accelerated to energies of tens or hundreds of kilovolts. Such sources were generally sufficient for studies of the crystals of comparatively small molecules, but collection of data for macromolecules such as proteins would often require many days or weeks. That situation changed radically only in the early 1970s with the introduction of synchrotrons as much more powerful radiation sources. In this paper we will review the early stages of the development of synchrotron radiation as a tool for structural biology in general, and protein crystallography in particular, and the various methodological advances that were the consequences of its application. Unlike in a number of previous reviews of this field (for example, see Cassetta *et al.*, 1999; Doniach *et al.*, 1997; Fourme *et al.*, 1999), we will not attempt to provide a complete and comprehensive historical record, but rather a personal story based largely on our own experiences during the early days of the utilization of synchrotron radiation for the studies of macromolecules.

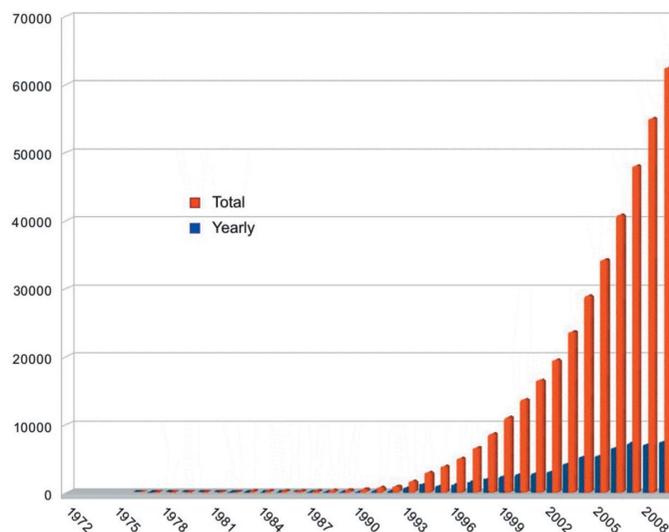
## 2. Brief history of early protein crystallography

Protein crystallography, perceived as a science dealing with protein crystals, can be traced as far back as 1840, with description of the serendipitously obtained 'blood crystals' of earthworm hemoglobin (Hünefeld, 1840). It is of note that in the first century of protein crystallization a gigantic volume of data on hemoglobin crystallization was published (Reichert & Brown, 1909), documented by 600 micrographs. The 1946 Nobel Prize in Chemistry went to James Sumner, John Northrop and Wendell Stanley, and was awarded essentially for crystallization of pure proteins and viruses.

As a structural science, however, protein crystallography is merely fifty years 'young', the stop-watch being set by the publication (Kendrew *et al.*, 1958) of the structure of myoglobin. Although the first model was created on the basis of very low resolution (6 Å) data, it was relatively soon followed by a structure with respectable 2 Å resolution (Kendrew *et al.*, 1960). Also, about the same time the structure of hemoglobin was unraveled (Perutz *et al.*, 1960). While Max Perutz, who started his titanic work on hemoglobin structure in 1937, was an unquestionable pioneer of protein X-ray crystallography, some work had been done even earlier. Specifically, in early 1930s the first protein X-ray diffraction images were recorded by William Astbury (Astbury & Street, 1932) for fibers and by J. Desmond Bernal for single crystals. The specimens used in the latter case were pepsin crystals, grown accidentally by John Philpot in Uppsala. Although the very first X-ray photograph was disappointing, Bernal quickly realised that excellent diffraction could be obtained if the crystals were prevented from dehydration (Bernal & Crow-

foot, 1934). In his famous remark, he observed that the X-ray pictures 'showed large unit cells with a great wealth of reflections found even at comparatively high angles corresponding to such low spacing as 2 Å [and that this] indicated that not only were the molecules of the protein substantially identical in shape and size, but also that they had identical and regular internal structure down to atomic dimensions' (Bernal, 1939). However, despite this brilliant start, progress in this field was initially very slow, as illustrated by the 22 years that it took Perutz to complete the structure of hemoglobin. With the next structures, things looked somewhat better, but still by 1971, when the Protein Data Bank (PDB) was created (Protein Data Bank, 1971), only seven protein structures were initially deposited (their number was increased by two by 1973, although a few more structures were by then solved, but not deposited). All those structures were determined by single-crystal X-ray diffraction. In addition to myoglobin and hemoglobin, the opening holdings of the PDB also included cytochrome  $b_5$ , basic pancreatic trypsin inhibitor (BPTI), subtilisin BPN', tosyl  $\alpha$ -chymotrypsin, carboxypeptidase A $\alpha$ , L-lactate dehydrogenase and rubredoxin (Protein Data Bank, 1973). It should not be overlooked that macromolecular fiber diffraction (in addition to Astbury's work on fibrous proteins) also produced at that time ground-breaking results, the most spectacular being the discovery of the double-helical structure of DNA by Watson & Crick (1953), supported by X-ray diffraction photographs recorded by Rosalind Franklin, Maurice Wilkins and others (Franklin & Gosling, 1953).

Obviously, the initial slow progress of protein crystallography in the period of its infancy (at least by the current standards) was a consequence of the stark lack of proportion between the Herculean goal and the less than humble means to achieve it. Among the inadequate experimental possibilities was the absence of high-brilliance sources of X-ray radiation, which made the crucial diffraction measurements extremely slow, if not impossible altogether in some cases. At first, improvement came in gradually, with better sealed-tube and then rotating-anode generators. However, in the 1970s, there was a dramatic qualitative improvement, which has changed the face of protein crystallography forever, as can be seen (with some time delay), for example, from the annual growth of PDB deposits (Fig. 1) and the current size of this database (Berman *et al.*, 2000). This 'quantum leap' was largely due to the introduction, at first greeted with caution, of X-radiation from synchrotron sources, followed by advances in protein engineering methods. According to an estimation by Gerd Rosenbaum, the increase of the X-ray flux in comparison with rotating anodes was 200-fold at the early synchrotrons, 8000-fold at the early storage rings, 80000-fold at the second-generation rings, 20 million times at the third-generation rings and  $6 \times 10^{17}$  times at the peak intensity of the X-ray laser source at Stanford. More accurate values for particular beamlines have been published previously (Helliwell, 1992, pp. 218–221). Of course, there were several other factors that have contributed to the boost that protein crystallography received at about the same time. Among the most important were the introduction of programmable high-speed electronic



**Figure 1**

Growth of the number of deposits in the Protein Data Bank. The number of annual deposits is shown in blue, and the cumulative total number in red.

computers and the use of recombinant methods for protein production. We will also mention them as additional circumstances in this article, although the main focus will be on the impact of synchrotron radiation on modern macromolecular crystallography.

### 3. The nature of synchrotron radiation

Circular accelerators of elementary particles were originally constructed as tools for high-energy physicists studying the subatomic structure of matter. In these installations the orbiting charged particles (*e.g.* electrons) were accelerated to relativistic velocity (close to the speed of light), thus gaining very high energy. Strictly speaking, synchrotrons are particle accelerators, and the practical sources of the 'synchrotron light' are the so-called storage rings, where particles are orbiting with constant energy. In practice, however, the term 'synchrotron' is often applied to a storage ring. The physicists then looked for novel subatomic particles, formed during collisions between the highly energetic electrons or ions. However, charged particles, when accelerated, emit electromagnetic radiation. From the point of view of the physicists, this was actually a nuisance because in this process the particles were losing energy, requiring constant 'energy pumping' and injection of more electrons. The synchrotrons and storage rings consist of evacuated rings and strong electromagnets, bending the trajectory of the electrons along closed, more-or-less circular, orbits. In practice they have the shape of polygons, with straight sections between the bending magnets. After injection and initial acceleration, the orbiting electrons have constant energy (and velocity), but undergo angular acceleration in the magnetic field of each of the bending magnets. The bending magnets are thus sources of electromagnetic radiation with a very wide range of wavelengths, from microwaves to hard X-rays and the possibility of

selecting an appropriate wavelength is particularly important for applications in protein crystallography. A detailed theory of this phenomenon was formulated already in the 1940s (Schwinger, 1949).

The early users of synchrotron radiation were treated by the principal synchrotron users (the high-energy particle physicists) as parasites. As discussed more fully below, that was the initial situation for protein crystallographers utilizing the synchrotrons in Hamburg, Stanford or Novosibirsk. However, when the full potential of synchrotron radiation for various scattering and diffraction experiments became fully appreciated, a new generation of synchrotrons were built, dedicated solely to the production of electromagnetic radiation. These second-generation machines were in fact not synchrotrons but particle storage rings with a circumference of the order of a few hundred meters, where high-energy electrons (or positrons) are injected and kept at a constant energy, usually about 2–3 GeV. The electron current (in the range of a few hundred mA) in these rings drops with time and every few hours more particles have to be injected. Such machines, SRS in Daresbury (UK), DORIS in Hamburg (Germany), LURE in Orsay (France), SPEAR storage ring at SSRL (Stanford, USA), Photon Factory in Tsukuba (Japan), CHESS at Cornell (USA) and NSLS at Brookhaven (USA), have been and still are very successfully used for sophisticated experiments, including macromolecular crystallography. In the straight sections between bending magnets, it is possible to place so-called insertion devices, wigglers or undulators. These are multipole magnets, where the orbiting particles are subjected to several ‘kicks’ of magnetic field, producing much stronger radiation than from ordinary bending magnets. In undulators, the radiation from each pair of magnetic poles interferes positively, leading to the emission of even stronger ‘spikes’ of X-rays at a certain wavelength, depending on the size of the gap between the poles.

The advantages of synchrotron radiation are not limited to high intensity and the possibility to select a desired wavelength from the wide spectrum of energies. The particles in the ring are grouped in bunches, and as a result the radiation is emitted in short pulses, lasting picoseconds, and separated by nanoseconds. This, together with the utilization of polychromatic white beam in Laue diffraction experiments, opens up the possibility of monitoring the progress of fast chemical processes taking place, for example, during enzymatic or other reactions (Bourgeois & Royant, 2005). Moreover, since the particles travel in a horizontal orbit, the emitted radiation is highly polarized with the electric vector in the equatorial plane, which can be utilized in certain experiments exploiting anisotropic effects (Schiltz & Bricogne, 2008). The application of synchrotron radiation in macromolecular crystallography has been comprehensively reviewed (Helliwell, 1992).

#### 4. Early development of synchrotron radiation sources for X-ray scattering studies

In Europe, the interest in the possibility of using synchrotron radiation as a research tool germinated first in the community

studying by diffraction methods the structural principles of muscle contraction. In those experiments, low-angle diffraction images were recorded from tiny ‘pseudocrystalline’ animal muscle fibers. In the late 1960s, two groups, one headed by Hugh Huxley in Cambridge, England, and the other by Ken Holmes in Heidelberg, Germany, were working on muscles from frogs and insects, respectively, using the relatively newly constructed rotating-anode sources of Cu  $K\alpha$  radiation. However, the muscle samples were very small and the diffraction effects were very weak. When in 1969 Gerd Rosenbaum, a physicist with some prior experience (as a student-diplomant in the UV spectroscopy group) with the already existing synchrotron at DESY (Deutsches Elektronen-Synchrotron) in Hamburg, started working with Holmes towards his PhD thesis on the construction of better X-ray sources for the muscle-contraction experiments, they directed their attention to the possibility of employing synchrotron radiation (Abad-Zapatero, 2004). Joined by Jean Witz of Strasbourg, they equipped an experimental hutch at DESY with a focusing quartz monochromator in vacuum, adjustable slits and other necessary parts. The use of this machinery was very tedious and involved a complicated procedure of setting interlocks and asking the central control (by telephone) to open the main shutter. This could be done only in the short intervals allocated to them between high-energy physics experiments. In the summer of 1970 they recorded the first diffraction images from insect muscle. The beam intensity was estimated to be a few hundred times higher than from a contemporary rotating-anode generator (Rosenbaum *et al.*, 1971).

After this initial success, Holmes and Rosenbaum were encouraged by the DESY Directors to construct, in a separate building, an experimental station dedicated to investigations of biological systems (Holmes & Rosenbaum, 1998). This attitude of Willibald Jentschke, Director of DESY, has to be appreciated, since the managements of other synchrotron facilities existing at that time were very reluctant to allow the ‘intruders’ to fiddle with their particle-physics machines. The new building, constructed in 1971, with direct connection to the main synchrotron ring, was (and still is) known as ‘bunker 2’, and contained more elaborate experimental facilities as well as some laboratory and office space.

In the early 1970s, John Kendrew, then head of the newly founded European Molecular Biology Organization (EMBO), realised that synchrotron radiation would play an extremely important role in the future of structural and molecular biology. He was also actively pursuing the initiative to establish the European Molecular Biology Laboratory (EMBL), a trans-national institution with a mission to conduct molecular biological research requiring international cooperation and funding. The application of synchrotron radiation fitted very well with these ideas. A formal agreement was reached between DESY and EMBL, and the experimental station in Hamburg became the key component of an official Outstation of EMBL. In 1975 it acquired a new building (bunker 4), located at one quadrant of the newly built storage ring DORIS.

At approximately the same time and in parallel, synchrotron radiation research was also pursued in the synchrotron facility operated by the Soviet Academy of Sciences in Novosibirsk. A comparatively simple station that utilized a graphite monochromator, but without any focusing capabilities, was used to obtain diffraction pictures from several small-molecule crystals, as well as from DNA fibers. It was also mentioned that diffraction pictures from crystals of a small protein, actinoxanthin, were obtained, but no additional details were provided (Mokulskaya *et al.*, 1974).

### 5. The first protein crystallography beamline at Stanford

Synchrotron radiation first became a practical research tool in the USA in May 1974, with the commencement of the operation of the SSRP (Stanford Synchrotron Radiation Project, later renamed SSRL), an experimental beamline built on the then recently completed SPEAR storage ring. The first beamline utilized 11.5 mrad of radiation from a bending magnet, divided into five separate experimental areas. Similarly to DESY with its emphasis on X-ray scattering, fiber diffraction was also the main reason for constructing beamline BL1-4. However, advancing protein crystallography was considered to be an important function of the new facility from the very beginning. Another technique that was being developed in parallel at SSRP was X-ray absorption or EXAFS (Lytle, 1999), but this technique, which only became practical with the use of synchrotron radiation, will not be discussed here any further.

Adaptation of the BL1-4 scattering station to enable protein crystallography experiments was the brainchild of Keith Hodgson, then a new untenured faculty member in the Chemistry Department at Stanford. The initial group working on the development of the station was quite small; it consisted of two postdoctoral fellows (Margueritte Yevitz Bernheim and AW) and a graduate student (James Phillips), with another postdoc, Julia Goodfellow, joining a year later. Since one of the authors of the current paper (AW) was there from the beginning, it is possible to give some personal reminiscences of the early days of the project.

The facilities of this first station were quite primitive. The only X-ray detector was an Enraf-Nonius precession camera that recorded diffraction images either on instant Polaroid films for alignment shots or on double-sided radiology films. The latter needed a darkroom for their development, but the nearest one was located on the main Stanford campus, at a distance of a couple of miles. This complication required frequent trips between the two sites in the 'official' vehicle of the SSRL, a Korean-war vintage armored personnel carrier donated by the US Navy. Thus, the three scientists involved (AW, James Phillips and Julia Goodfellow) had to drive it to and from the campus; with none being at that time American citizens, it was rather amusing.

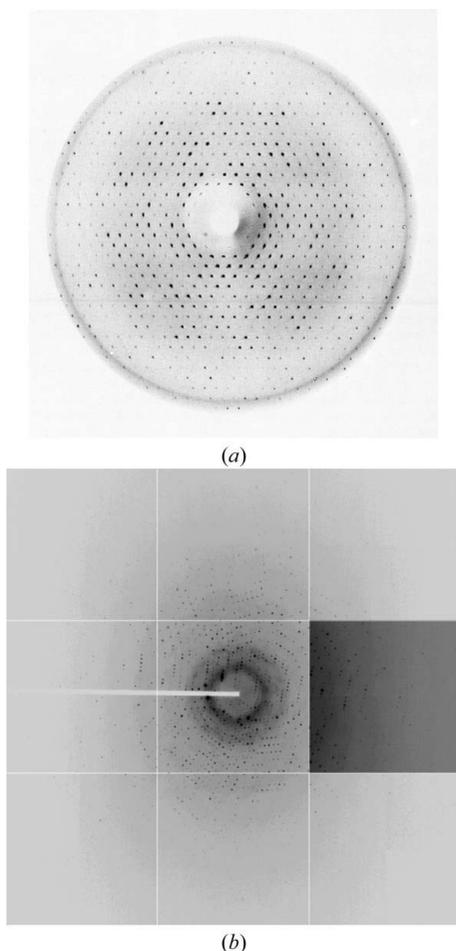
The necessity to travel between the locations led us to missing one of the most exciting moments at SPEAR, when a major fire broke out in the experimental hall used by the

physicists. We had just gone to the main campus to develop the first precession film taken for a crystal of hen egg-white lysozyme, the protein commonly used for development of new crystallographic techniques and methods. We were delighted by the presence of some faint spots that indicated that the crystal did diffract, but when we returned to continue the experiments we were surprised to find the place in total darkness. Fire engines had just left, and for the next six weeks it was not possible to do any further work.

A major problem faced not only by crystallographers but also by all scientists that needed to utilize hard X-rays was connected with the parasitic nature of the operations; the physicists were firmly in charge of setting the operating parameters of the ring. Their work resulted only two years later in a Nobel Prize for Burton Richter. Unfortunately, the psi particles that he discovered were found when the beam energy was set to 1.55 GeV, too low to produce useable X-rays. The second particle discovered by the physicists, psi prime, was not much better, since it required operation at 1.86 GeV, whereas for useful production of X-rays the ring energy had to be at least 2.0 GeV. The success of the physicists delayed synchrotron radiation work by quite a few months.

The first results came after a rather long time and much effort, and only provided a proof of principle rather than data that would be useful on their own (Phillips *et al.*, 1976). Of the four proteins for which some data were collected, only one, azurin, yielded a three-dimensional data set, whereas only a single central layer was recorded for the other three (rubredoxin, nerve growth factor and L-asparaginase). Incidentally, the crystal structures of the latter two proteins were solved only after another 15 years! The exposure times were as long as five hours in the case of nerve growth factor, but, since no significant diffraction data from similar crystals could be obtained using a standard X-ray source, even that result was considered to be a success. In addition, even at that early stage it was understood that synchrotron radiation might offer the best opportunity to measure data at multiple wavelengths, circumventing the need for heavy-atom derivatives, at least for some crystal structures (Herzenberg & Lau, 1967).

Further progress was achieved when a collaborative study with the group of Lyle Jensen showed the feasibility of extracting at least some phase information from careful analysis of the diffraction data of rubredoxin. That protein was chosen since it contained a covalently bound iron, and with the absorption edge at 1.75 Å it was possible to maximize the anomalous effect. Another useful property of rubredoxin crystals was their space group, *R*3, one of very few in which the central projection is non-centrosymmetric and contains non-zero Bijvoet differences even on the *hk0* precession photograph (Fig. 2a). Thus, it was possible to locate the iron ions, at least in a projection, based on diffraction data recorded on a single film (Phillips *et al.*, 1977). Some technical problems not encountered before had also to be solved; for example, calculation of the polarization correction, necessary since the synchrotron beam has the electrical vector polarized in the equatorial plane. The experiments were by no means easy or short. Since the crystals had to be aligned using Polaroid films,



**Figure 2**

Protein diffraction collected at synchrotron sources, then and now. (a) Precession photograph of the  $hk0$  layer of a rubredoxin crystal, taken at the Stanford SSRL storage ring in 1975. The exposure time was 5 h. Careful visual analysis shows differences in intensities of the Friedel mates. (b) Diffraction image from a trichloroethylene crystal of hen egg-white lysozyme taken on beamline 19ID at APS, Argonne, IL, USA. The white lines are due to dead regions between the nine separate panels of a MAR300CCD camera. The image in the middle panel on the right is displayed in enhanced contrast, showing that measurable reflections extend to the edge of the detector at a resolution of 0.65 Å. The exposure time was 5 s with the beam attenuated to 30%.

it was necessary to open the experimental hutch every few minutes. Whereas the hutch was supposed to prevent the experimenters from coming too close to the X-ray beam, we found ways to enter it, at least for the purpose of getting photographed (Fig. 3). With the beam life time of the order of 2 h, that was the longest period available for sleep for the scientists involved. The longest single experiment took five days and six nights, resulting in complete exhaustion of the participants. We were highly motivated, since we knew that a Soviet group working in Novosibirsk was also developing a synchrotron station for protein crystallography (Mokulskaya *et al.*, 1974), and, until our first paper was published, we were always afraid of being scooped. This did not happen, however, since our competitors did not publish their results, but it provided additional strong motivation for our work.



**Figure 3**

A hutch of the first macromolecular crystallography beamline 1–4 at Stanford that was supposed to prevent scientists from getting too close to the beam. From the left: Marguerite Yevitz Bernheim, Keith Hodgson, AW and James Phillips. Of course, the beam was closed when this picture was taken!

## 6. Protein crystallography at DESY

The first attempts to use synchrotron radiation for protein crystallography in Hamburg by the group of Georg Schultz (Harmsen *et al.*, 1976) were somewhat discouraging, since the beam from the synchrotron was not stable enough, in contrast to the situation at Stanford where the beamline was constructed on the storage ring. The situation changed with the construction of the DORIS storage ring. For the first two decades of its service, DORIS operated in two modes. It either served mainly high-energy physics experiments, with both electrons and positrons orbiting in the ring in opposite directions, or it was dedicated to the production of synchrotron light with only electrons in the ring.

After some time, the EMBL Outstation was the owner of five beamlines: three located in the main DORIS experimental hall (HASYLAB) and exploiting the electron current, and two in its own bunker 4, based on the positron current. The latter lines were useable only during ‘parasitic time’, when DORIS was filled with positrons, but they were much more ‘private’, with the HASYLAB safety people very rarely visiting. The beamlines located in HASYLAB served SAXS (X33), EXAFS (X32) and macromolecular crystallography (X31), while the bunker 4 lines were used for crystallography (X11) and SAXS (X13) experiments. The X31 line operated on a bending magnet and used a channel-cut monochromator. It was, therefore, easy to change the wavelength of the X-ray radiation. In contrast, X11 had a single triangular monochromator, so changing wavelength was much more difficult and, therefore, rarely executed. However, the X-ray beam from X11 was significantly stronger than from X31. Both end-stations had movable cradles supporting a collimator, a goniostat and an X-ray camera, which were controlled remotely. Darkrooms were located in the vicinity of the stations, because until the end of the 1980s all data were collected on photographic films, using the 8-cassette Arndt-Wonacott Enraf-Nonius rotation camera.

The protein crystallography group at EMBL Hamburg was headed first by Hans-Dieter Bartunik, who later had an

independent unit of the Max-Planck Institute at DESY and managed the new BW6 beamline. Between 1985 and 1996, the head of the EMBL group was Keith Wilson, initially with three staff scientists (Kyriacos Petratos, Christian Betzel and ZD) and later by Victor Lamzin and Matthias Wilmanns with several additional staff members.

The loading of cassettes with packs of three films (sometimes interspersed with aluminium or steel foil as attenuators at short wavelengths), marking them in pencil and placing fiducial marks was a full-time job for one of the (unlucky) experimenters. Developing, fixing and washing hundreds of films in almost complete darkness and loading cassettes on the carousel usually required another pair of hands during experiments lasting sometimes more than one day and night. It remains unknown how many shirts and trousers of people too keen to inspect newly exposed films directly from the developer bath were ruined by acid stains.

The work with films involved sometimes some interesting, not always happy, events. One of the staff scientists could hardly refrain from laughing when one of the young students (sent from a prominent laboratory to collect data single-handed) carefully 'developed' the black interspersing papers and threw away to the wastebasket all the exposed films! Unexpected events happened also to very distinguished scientists. Ada Yonath, always very energetic and keen to do things fast and efficiently, wanted on one occasion to wash the developed films very quickly and ran the water stream in the darkroom tank at full blast. One of the films got loose and blocked the sink drain. As a result, the floor of the main hall of HASYLAB was very quickly under water.

Injections of particles into the storage ring took place every two to three hours. After each injection, all shutters had to be opened manually. This often collided with the staff lunch or dinner hours. At X31, under the watchful eyes of the HASYLAB safety personnel, the procedure was always strictly observed. At the more 'private' EMBL X11 line, the red button, for manual activation of the shutters, was sometimes permanently depressed by an ingenious machinery consisting of a long ruler and a cork, while the staff people enjoyed their time in the local 'library' (a bistro or pub in the vicinity of DESY). Such an illegal procedure should certainly not be condoned, and, of course, would not be possible nowadays.

The life of crystallographers using the EMBL beamlines (especially of staff scientists) vastly improved in 1989, when Jules Hendrix and Arno Lentfer (then EMBL staff members) constructed an automatic on-line imaging plate (IP) scanner, the predecessor of all later MAR IP detectors. No more time spent in darkrooms and no more toying with the optical film scanner! The prototype red-colored detector was adored by everybody. The first days of its use were not without surprises, though. The very first data were collected with this machine by Alex Teplyakov on a crystal of thermitase. After spending a night at X31, he complained that each exposure required a separate file name (differing just by a sequence number) that had to be typed in. Of course, the next day Michael Böhm, who had written the scanner control software, modified the

program and the computer automatically increased the image number, relieving the experimenter's fingers of this task. However, close inspection of highly zoomed diffraction images revealed a troubling fact: quite suspiciously often, pairs of adjacent pixels had exactly the same value of intensity. The explanation was found after a short talk to Michael. An expert in pattern recognition, he transformed the original spiral scan of the detector pixels into the nearest Cartesian pixels for subsequent interpretation, and overlooked the fact that often one spiral pixel had two closest Cartesian neighbors! Surely enough, the next morning the algorithm was modified and the mapping was not only faithful with respect to shape (as required by shape imaging) but also correct numerically. A potentially more serious problem arose when the first newly obtained experimental Fourier map of dUTPase, phased using anomalous data of a mercury derivative, revealed that although all main-chain atoms very happily agreed with the electron density, all  $C\beta$  atoms were sticking out of density. Again, a discussion with Michael resulted in a reversal of the way the detector files were written out, and from then on all data sets had the correct chirality.

The DORIS ring underwent a major reconstruction in 1989. The high-energy physics experiments were discontinued, and the ring became a dedicated synchrotron light source. Only positrons were orbiting now the ring, in the direction of the previous electron current, and this required inverting of the X11 (and X13) beamlines in the opposite direction. At the same time, the new HASYLAB hall was built, accommodating not only X11 and X13 but also the newly constructed wiggler beamlines BW7A and BW7B. Another wiggler beamline, BW6, was constructed and supervised by Hans-Dieter Bartunik from the Max-Planck Institute. This is still more or less the current situation, plus a few upgrades and improvements. For instance, most of the imaging plate scanners have been replaced by CCD detectors. In the near future, however, the EMBL Outstation in Hamburg will start operating several brand new beamlines at the newly reconstructed large PETRA III ring, which at an energy of 6 GeV will deliver much brighter X-ray beams.

### 7. Multiwavelength anomalous diffraction (MAD), a technique tailored for synchrotron radiation

The possibility to tune the wavelength of synchrotron radiation to the  $\lambda$  values that are precisely optimal for a given experiment is probably one of the most exceptional features that has revolutionized the way macromolecular structures are now solved. At the core of this issue is the notorious crystallographic phase problem, which precludes a straightforward calculation of the structure from the experimental diffraction intensities alone, but requires that for each of those numerous intensity data an additional phase term must be estimated. If there is no suitable model for use in the method of molecular replacement, then those missing phase terms must be estimated experimentally. The classic method of isomorphous replacement (MIR) invented by Perutz requires collecting additional diffraction data sets from isomorphous derivative

**Table 1**

Synchrotron beamlines in current use for macromolecular crystallography.

ALS (Advanced Light Source), Lawrence Berkeley National Laboratory, Berkeley, USA	4.2.2, 5.0.1, 5.0.2, 5.0.3, 8.2.1, 8.2.2, 8.3.1
APS (Advanced Photon Source), Argonne National Laboratory, Argonne, USA	14BM-C, 14BM-D, 14ID-B, 17BM, 17ID, 19BM, 19ID, 21ID-D, 21ID-E, 21ID-F, 21ID-G, 22BM, 22ID, 23BM-B, 23ID-B, 23ID-D, 24BM, 24ID-C, 24ID-E, 31ID
Australian Synchrotron, Melbourne, Australia	MX1, MX2
BESSY II, Berlin, Germany	14.1, 14.2, 14.3
BSRF, Beijing, China	3W1A, 1W2B
CAMD (Center for Advanced Microstructures and Devices), Baton Rouge, USA	GCPC
CHESS (Cornell High Energy Synchrotron Source), Cornell University, Ithaca, USA	A1, F1, F2
CSRF (Canadian Synchrotron Radiation Facility), Saskatoon, Canada	08ID-1
DIAMOND, Harwell Chilton Science Campus, England	I02, I03, I04, I04-1, I24
ELETTRA, Trieste, Italy	5.2R
EMBL/MPG (European Molecular Biology Laboratory, Max-Planck Gesellschaft DESY), Hamburg, Germany	BW7A, BW7B, X11, X12, X13, BW6
ESRF (European Synchrotron Radiation Facility), Grenoble, France	ID14-1, ID14-2, ID14-4, ID23-1, ID23-2, ID29
LNLS (National Synchrotron Light Laboratory), Campinas, Brazil	D03, W01B
MAX, Lund University, Sweden	I711, I911-2, I911-3, I911-4, I911-5,
NSLS (National Synchrotron Light Source), Brookhaven National Laboratory, USA	X3A, X4A, X4C, X6A, X8C, X12B, X12C, X25, X26C, X29A
NSRRC (National Synchrotron Radiation Research Center), Taiwan	BL13B1, BL13C1, BL17B2
PAL, Pohang, Korea	4A, 6B, 6C1
Photon Factory, Tsukuba, Japan	BL-5A, BL-6A, BL-17A, BL18-B, AR-NW12A
SLS (Synchrotron Light Source), Paul Scherrer Institute, Villigen Switzerland	X06SA, X10SA, X06DA
SOLEIL, Saint-Aubin, France	PROXIMA1, PROXIMA2
SPring-8 (Super Photon Ring 8), Japan	BL12B2, BL24XU, BL26B1, BL26B 2, BL32B2, BL38B1, BL40B2, BL41XU, BL44XU, BL45XU
SSRL (Stanford Synchrotron Light Source), Stanford University, USA	BL1-5, BL7-1, BL9-1, BL9-2, BL11-1, BL12-2

crystals, in which the protein molecule has been labeled by very heavy electron-rich atoms. [Classic heavy ions are metal cations, but a variant exists that exploits halide anions (Dauter *et al.*, 2000).] It was realised early on that atoms with suitable electronic configuration can mark their presence in a protein crystal not only by their high electron count but also by resonant absorption of the X-ray quanta, leading to the dependence of their scattering factors on  $\lambda$ ,  $f = f^o + f'(\lambda) + if''(\lambda)$ , a phenomenon known as anomalous dispersion. Although anomalous dispersion has been often used as an auxiliary source of phasing information in macromolecular crystallography, its full application with home sources of X-ray radiation was generally not possible because of the sporadic coincidence between the available wavelengths (*e.g.* Cu  $K\alpha$  1.5418 Å) and the characteristic absorption edges of typical MIR elements.

The situation changed radically when synchrotron beamlines with tunable wavelength offered a solution. The possibility to adjust the wavelength is in reality an extension of the experimental set-up of any monochromatic synchrotron beamline, and it consists of the ability to change the monochromator angle in order to select a desired  $\lambda$ . Modern tunable beamlines use double monochromators with parallel glancing surfaces (or a single monochromator crystal with a channel cut through it) which guarantees that the monochromated beam emerges from the optical device in the same direction regardless of the monochromator angle. Other experimental challenges include the requirements for very high precision and reproducibility, both in wavelength and in geometrical parameters. Yet another requirement is very high accuracy of the intensity measurements, as the anomalous effects are usually quite small, but this aspect is important at any synchrotron beamline. These technicalities have been

successfully solved and there are quite a number of tunable synchrotron beamlines in operation (Table 1).

In a typical experiment designed to exploit anomalous effects, complete X-ray diffraction data sets are collected at wavelengths selected to maximize the  $f''$  and  $f'$  effects (absorption peak and absorption edge, respectively), and usually at one or more additional  $\lambda$  values, away from the absorption edge. The use of multiple wavelengths has given the method its name, multiwavelength anomalous diffraction, or MAD [see Hendrickson (1999) for a more detailed review]. This method was introduced into macromolecular crystallographic practice through a series of papers published between about 1985 and 1990 (Guss *et al.*, 1988; Harada *et al.*, 1986; Hendrickson *et al.*, 1990, 1991; Korszun, 1987; Yang *et al.*, 1990) although the possibility of measurements at multiple wavelengths had been recognized much earlier (Herzenberg & Lau, 1967; Mitchell, 1957; Okaya & Pepinsky, 1956). The theoretical foundations of the MAD method were formulated by Jerome Karle (Karle, 1980) and then developed into a practical algorithm by Wayne Hendrickson (Hendrickson, 1985). According to this formalism, the  $\lambda$ -dependent and  $\lambda$ -independent contributions to scattering can be separated in a set of equations, which can then be solved algebraically, giving in the end the desired reflection phases. Since, in the MAD method, physics (change of wavelength) rather than chemistry (exchange of the heavy atom for a different species) is the source of phasing information, all the multiple experiments are performed using the same crystal, which is an additional strength of this method, greatly simplifying the experimental procedures and enhancing accuracy.

The first tests of the MAD method with synchrotron radiation, conducted for known crystal structures, were reported for terbium-derivatized parvalbumin (Kahn *et al.*,

1985), for the iron-containing cytochrome *c'* (Harada *et al.*, 1986) and lamprey hemoglobin (Hendrickson *et al.*, 1988), and for copper-containing azurin (Korszun, 1987). Probably the first protein structure determined completely *de novo* with MAD was of the copper-containing protein CBP. Crystals of CBP were available for over a decade, yet the structure could not be solved by any other means (Guss *et al.*, 1988). However, the anomalous scatterers utilized in these pilot studies, while scientifically interesting, were not amenable to automated routine application because either a tedious derivatization process was involved (with terbium as an example) or the rather rare situation of a suitable native constituent (iron or copper) was exploited. The fact that in the MAD approach the wavelength is precisely tuned to resonance with the anomalous scatterer makes this method applicable also to elements with relatively small anomalous effects, provided their absorption edge lies within experimentally accessible X-ray wavelengths. In practice, all elements at least as heavy as chromium are good candidates for MAD experiments. Thus, useable MAD elements do not have to be literally heavy at all, and one particularly useful element is selenium, with its anomalous effects of about 5 and  $-9$  electrons for  $f''$  and  $f'$ , respectively. These two effects occur at  $\lambda$  values that differ by as little as  $0.0002 \text{ \AA}$  (equivalent to  $2.5 \text{ eV}$ ) near  $\lambda = 0.979 \text{ \AA}$ , which illustrates the demanding experimental conditions regarding the precision of wavelength selection and its reproducibility.

Introduction of the use of selenium as an anomalous scatterer was a major breakthrough, establishing MAD as a technique of choice for solving new protein structures. The attractiveness of selenium lies in the fact that it can be relatively easily incorporated into protein sequences as selenomethionine (Se-Met) in place of the natural sulfur-containing amino acid methionine (Met) (Hendrickson *et al.*, 1990). Today, thanks to advances of protein engineering, this incorporation is a matter of routine, also possible with automated protein production methods. If a bacterial strain (usually *Escherichia coli*) used as a factory for recombinant protein production is a methionine auxotroph (*i.e.* is methionine-dependent) and the culture medium contains Se-Met instead of Met, the newly synthesized protein will be labeled with selenium in all sequence positions occupied by methionine. It is also possible to incorporate Se-Met using ordinary bacteria, in which the Met biosynthetic pathway has been blocked. Since, statistically, Met occurs with about 2.5% frequency, usually there are enough Se atoms for successful application of MAD phasing. While Se-Met MAD is today thriving as a high-throughput method of choice for the investigation of proteins with novel folds, originally it was introduced cautiously as a scientific curiosity. In fact, the first protein structure solved by Se-MAD phasing was streptavidin in complex with the vitamin biotin (Hendrickson *et al.*, 1989), whose single sulfur atom was chemically replaced with selenium. Perhaps the first true triumph of the classic Se-Met MAD approach was the structure of ribonuclease H (Yang *et al.*, 1990), solved to elucidate the complete machinery of the HIV reverse transcriptase complex.

Bromine, which can be used to modify, with minimal chemical consequences, the nucleobases in nucleic acids structure, can play in crystallographic studies of DNA and RNA a role similar to selenium in protein crystallography. Even more attractively,  $\text{Br}^-$  can be incorporated into macromolecular crystals by a quick soak (Dauter *et al.*, 2000), making Br-MAD another possibility for high-throughput biological crystallography at synchrotron beamlines.

Although, with classical MAD, data sets collected at least at two wavelengths are necessary for the algebraic solution of the phase problem, a simpler approach that utilizes only one wavelength (SAD) is becoming increasingly popular (Dauter *et al.*, 2002). The success of SAD is possible because of the high accuracy with which synchrotron diffraction data are nowadays measured. It is then possible to extract the weak phasing signal even if it is submerged in a high level of noise.

Utilization of the MAD technique has not always been routine. In 1994, a battle was fought to crack the structure of retroviral integrase. When all other means had failed, the laboratory of MJ and AW turned to Se-Met MAD and sent a team with crystals of the catalytic domain of avian sarcoma virus (ASV) integrase to the only facility in the US that offered a more-or-less routine MAD environment, at the F1 beamline of the CHESS synchrotron. When, after a very brief introduction on Friday afternoon, we were left to our own devices, we realised that the qualification 'routine' was only a very approximate term. The beamline was controlled by a rather ancient computer which would hang quite frequently, only aggravating the interruptions caused by the physics experiments, for which the synchrotron was primarily used at that time. The entries in the logbook left by the previous users suggested very long exposure times ( $\sim 20 \text{ min}$ ) but following those examples we would never collect a complete MAD dataset. A bold decision was thus made to collect 20–30 s frames. During one of the computer failures the worst thing happened: we lost count of the peculiar naming sequence of the frames. It took us about half a day to figure out what was what and how to rename the files in proper sequence. The images turned out to be quite stable and in the end we did solve the structure (Bujacz *et al.*, 1995). It appears that it was one of the first (if not the first) successful MAD experiments at that beamline by external users.

## 8. Synchrotron radiation as the leading edge of new methods

A number of new methods that have led to improved quality of crystallographic data were developed as corollaries to synchrotron radiation experiments. One of the important developments that resulted in vast improvement in the ability to collect such data was the introduction of routine cooling of crystals to a temperature of  $\sim 100 \text{ K}$ , meant to reduce the heat load generated by the intense X-ray beam and to decrease the radiation-induced decay of the sample. Although cryocrystallography has been attempted on and off since the inception of the technique, it was made practical by Håkon Hope only at the end of the 1980s (Hope, 1988). One of the first practical

applications of cryocrystallography of proteins was in the studies of ribosome crystals (see below) (Hope *et al.*, 1989). The use of cryogenic temperatures, which technically involves a stream of gas nitrogen refrigerated to about 100 K, has in turn entailed a completely new method of mounting crystals for X-ray diffraction experiments. Today, the use of sealed capillaries with a clumsy drop of mother liquor is almost forgotten. Instead, protein crystals are suspended in a thin film of mother liquor in a miniature lollipop cryoloop, which is inserted in the cold jet for flash vitrification (Teng, 1990). To prevent ice and salt crystallization, special cryoprotectants (*e.g.* glycerol) are used and the nuisance of solvent background is greatly reduced. While originally cooling was applied only during X-ray exposure, today it has also become a routine method for protein crystal storage and shipping.

We (MJ and AW) introduced cryocrystallography in our laboratory quite early, although not everything was easy from the first moment. Our experiments with ASV integrase were being done in the era when cryogenic measurements were just starting to be routine. For example, no standard tools for cryobiocrystallography were then available and we had to design and manufacture (using proper blueprints and a precision mechanical workshop) our own magnetic crystal mounting pins. Very similar pins were later commercialized, for instance by Hampton Research. From some experienced colleagues we heard that flash cooling in a gaseous nitrogen stream was bad practice, and that the crystals should be frozen in a liquid medium but that it was extremely important to first use liquid propane and only then liquid nitrogen. Equipped with this knowledge, we purchased a 25 l dewar of liquid propane and traveled 500 km to the synchrotron at Cornell with this vessel in a passenger car! Fortunately none of us smoked and we had the laboratory fire marshal as a member of the experimental team.

The high flux of X-rays generated by synchrotron sources can lead to a rapid destruction of the crystalline order, thus limiting the resolution of the recorded diffraction. Chemically, the culprits are the free radicals generated by the ionizing radiation, which propagate throughout the crystal degrading the delicate protein material. As mentioned above, cryocrystallography was introduced as a way to counteract this phenomenon, but the problem of radiation damage reappeared at very bright third-generation synchrotron beamlines. Cryo-cooling mitigates the secondary radiation damage, arresting propagation of active radicals inside crystals, but cannot stop the effects of primary damage, *e.g.* when chemical bonds are broken by direct absorption of X-ray quanta. The first effects of damage are manifested by some localized specific structural changes, such as disrupted disulfide bridges or decarboxylation of glutamates and aspartates (Weik *et al.*, 2000; Helliwell, 1988). Ultimately, even cryo-cooled protein crystals completely lose the ability to diffract X-rays after sufficiently long exposure.

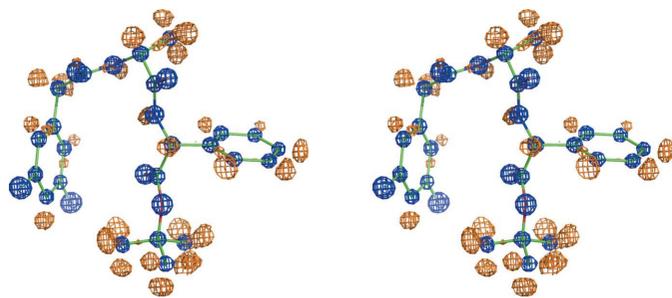
There is, however, an optimistic aspect of this situation. These destructive effects can be used in a positive sense for phasing novel structures by a technique appropriately named RIP (radiation-damage-induced phasing) (Ravelli *et al.*, 2003),

or, with an anomalous scattering component, RIPAS (Zwart *et al.*, 2004), where the intensity differences resulting from the structural changes are treated as isomorphous signals in analogy to the MIR or MIRAS approaches.

A branch of protein crystallography that is feasible exclusively with high-brilliance synchrotron sources is the Laue method, where a stationary crystal is exposed to a wide spectrum of non-monochromated (white) radiation. It is then possible to record an almost complete diffraction data set in a single, very short (nanoseconds) exposure. This approach has been pioneered for protein crystals by Keith Moffat (Moffat *et al.*, 1984), John Helliwell (Helliwell *et al.*, 1989) and Janos Hajdu (Hajdu *et al.*, 1987). Although this method has some theoretical limitations, and has never been used for solving novel protein crystal structures, it can be applied to study the structure of short-lived species, such as enzymatic reaction intermediates, or to follow kinetic transformations in protein crystals, *e.g.* the dissociation paths of ligands, such as CO in myoglobin (Milani *et al.*, 2008). If a chemical process within a protein crystal can be triggered, for example by a laser flash, then, by taking nanosecond shots at microsecond intervals, one can map the path of the reaction in a time-interval of, say, milliseconds.

Without synchrotron radiation it would not be possible to obtain atomic resolution (defined as 1.2 Å) or especially ultrahigh-resolution diffraction data from protein crystals (Fig. 2*b*). At present, there are about 1000 macromolecular structures in the PDB with resolution exceeding 1.2 Å, including about 20 sets with data beyond 0.8 Å, all of which were measured using synchrotron radiation. Ultrahigh-resolution structures are like gemstones to structural biologists because they allow individual atoms to be pinpointed without ambiguity as isolated peaks in electron density maps and even to visualize bonding electrons and the weak signals produced by H atoms. This latter aspect is of great importance because H atoms are usually crucial for the understanding of enzyme mechanisms but cannot be reliably located at lower resolution. Currently, the record resolution in the PDB, 0.54 Å, has been set for a small protein (46 residues) called crambin (Jelsch *et al.*, 2000). However, in the record-breaking zone there are also larger proteins, for instance lysozyme (129 residues) (Wang *et al.*, 2007) or aldose reductase (316 residues) (Howard *et al.*, 2004) characterized at 0.65 and 0.66 Å resolution, respectively. At such a level of detail the record-setting macromolecular structures not only attain but, indeed, surpass the standard typical of small-molecule crystallography (Fig. 4). In two most exciting studies, crambin (Jelsch *et al.*, 2000) and aldose reductase (Guillot *et al.*, 2008) have been refined using a multipole model, which essentially analyzes the distribution of bonding electrons and the deformation of atomic charge distribution from the normally assumed spherical approximation. This unprecedented level of insight, possible thanks to synchrotron radiation, opens up a completely uncharted area in the structural analysis of macromolecules.

Structural genomics, aiming at solving the largest number possible of novel protein structures in the least amount of time, is another area that has been crucially dependent on the



**Figure 4**  
Stereoview of the structure of DAPT, an oligopeptide inhibitor of  $\gamma$ -secretase, refined at 0.7 Å resolution using synchrotron radiation data. The blue electron density corresponds to non-H atoms and the brown difference map reveals all H atoms, missing from the model at this stage. It is of note that all methyl groups have well defined conformation. The figure was provided by courtesy of Andrzej Czerwinski.

availability of synchrotron sources for data collection. Some structural genomics centers solve, on average, one new protein crystal structure every other day (Grabowski *et al.*, 2009), and the amount of data required for that purpose is so vast that no traditional X-ray sources would be capable of providing it.

An area of studies of vast importance to the pharmaceutical industry, and therefore to ordinary people, that owes its existence to the availability of synchrotron radiation involves lead discovery, and optimization and refinement of the structures of potential drugs (Blundell *et al.*, 2006). Such studies often involve analyses of hundreds of complexes of target molecules with small inhibitors or their fragments, or even with cocktails of small molecules that can guide the synthesis of more potent molecules. Such approaches would not be feasible without the almost unlimited amount of X-rays that can be tapped at synchrotron sources.

## 9. 'To boldly go where no one has gone before'

A number of truly incredible scientific achievements would not have been possible without utilization of synchrotron radiation. For example, diffraction images were recorded and interpreted from crystals with staggering unit-cell dimensions of 1255 Å, in the case of *F432* crystals of reovirus core [PDB code 1EJ6 (Reinisch *et al.*, 2000)], or 1135 Å, in the case of *P3212* crystals of the clathrin adaptor protein [PDB code 1W63 (Heldwein *et al.*, 2004)]. The use of a synchrotron microbeam made it possible to collect diffraction data to 2 Å resolution from crystals of cypovirus polyhedra, naturally occurring in host insect cells and measuring as little as 2 µm (20000 Å) across (Coulibaly *et al.*, 2007).

Last but not least, the use of synchrotron radiation was critically important for a number of scientific accomplishments awarded with Nobel Prizes. This subject was covered in considerable detail ([http://nobelprize.org/nobel\\_prizes/chemistry/laureates/2009/press.html](http://nobelprize.org/nobel_prizes/chemistry/laureates/2009/press.html)) in the official description of the scientific background of the 2009 Chemistry Prize awarded to Venkatraman Ramakrishnan, Thomas Steitz and Ada Yonath for the determination of the atomic structure and mechanism of the ribosome based on high-resolution crystal

structures (Ban *et al.*, 2000; Schluenzen *et al.*, 2000; Selmer *et al.*, 2006; Wimberly *et al.*, 2000). During almost 30 years of studies of ribosome crystals by Ada Yonath and about 15 years by the other two groups, these scientists have used almost every synchrotron beamline available to them. A number of previous Chemistry Prizes were also awarded for seminal achievements in structural biology that relied on extensive utilization of synchrotron radiation. Probably the earliest such prize was awarded to John Walker in 1997 for the elucidation of the enzymatic mechanism underlying the synthesis of ATP (Abrahams *et al.*, 1994; Abrahams & Leslie, 1996). The diffraction data for  $F_1$ -ATPase crystals were measured at the SRS in Daresbury (UK) using the first on-line image plate outside Hamburg, a gift from Jules Hendrix and Arno Lentfer. The data needed to solve the structure were collected using many crystals mounted in capillaries and cooled to 277 K. Other prizes for research that relied on diffraction data collected using synchrotron radiation were awarded to Roderick MacKinnon in 2003 for his work on the structure and action of the membrane-embedded potassium channels (Jiang *et al.*, 2002) and to Roger Kornberg in 2006 for the elucidation of the structural basis of DNA transcription (Cramer *et al.*, 2001).

## 10. Future prospects of the application of synchrotron radiation in protein crystallography

In the 1990s the advances in technology led to the construction of third-generation synchrotron sources, characterized by larger ring sizes (diameter of ~1 km) and much higher beam brightness and stability. Such machines were first built in Grenoble, France (ESRF), Chicago, USA (APS; Fig. 5a), Harima Science Park City, Japan (SPring-8) and Berkeley, USA (ALS), and other recent examples include synchrotrons in Didcot, UK (DIAMOND), Saint-Aubin, France (SOLEIL), Villigen, Switzerland (SLS) and Hamburg, Germany (PETRA III). Construction of these new storage rings brought the number of synchrotron beamlines available for macromolecular crystallographic diffraction experiments (Fig. 5b) to about 100, with several more being built or planned (Table 1). Synchrotron radiation is now routinely utilized for collecting diffraction data in macromolecular crystallography, with a vast majority of new structures obtained that way (Fig. 6).

Even more sophisticated fourth-generation linear X-ray laser sources, like the one already operating at Stanford (USA) or being constructed in Hamburg (Germany), are able to provide about ten orders of magnitude brighter X-ray beams for single-shot diffraction experiments, opening up the possibility of performing novel experiments, such as investigating structures of large biological complexes, whole cells or imaging non-periodic nanostructured materials (Bergh *et al.*, 2008). In an X-ray free-electron laser (XFEL), the electron beam is not circulated in a ring but instead is accelerated to relativistic energies in an open-ended device, up to several kilometers long, through a long single-pass undulator. Since the electrons are in resonance with the electromagnetic field that they have produced, a lasing effect and an amplification of



(a)



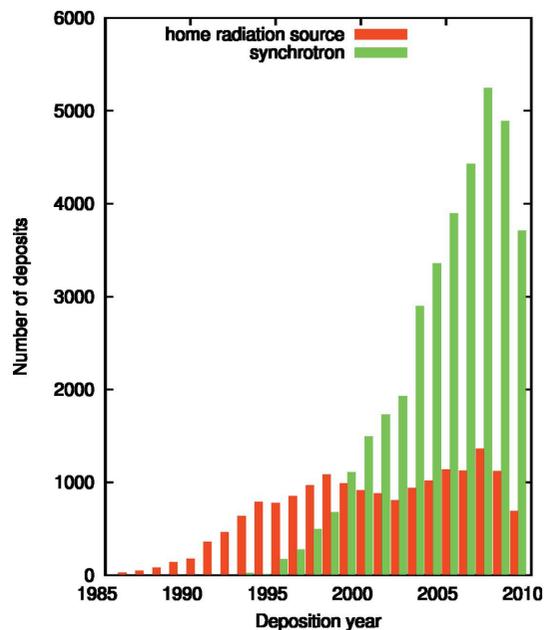
(b)

**Figure 5**

Modern synchrotrons and data collection stations. (a) Aerial view of the third-generation synchrotron ring of the Advanced Photon Source (APS) at Argonne, IL, USA. The ring consists of 40 sectors with a circumference of 1104 m. (b) The macromolecular crystallography experimental end-station 22ID at the APS used by members of the Southeast Regional Collaborative Access Team (SER-CAT). Photograph by John Gonczy.

light is possible. An important characteristic of XFELs is their very short pulse, down to a few femtoseconds. This is expected to be critical for avoiding radiation damage in diffraction experiments on single molecules and small clusters. The increase of the power of the X-ray beam in these new devices is so staggering that it is not even completely certain at present if the lifetime of biological samples in the beam will be sufficient to collect all the necessary data. However, such problems have been successfully solved in the past, so it is likely that they will be also properly handled in the future. The revolution caused by the introduction of synchrotron radiation as a tool for structural biology is not yet over. It may be expected that, also in the future, use of synchrotron radiation will contribute to even more awesome discoveries in structural biology, leading to better understanding of the processes of life at the atomic level, with beneficial consequences for human health and well being.

We are grateful to our colleagues who shared with us their stories and early experience as synchrotron radiation users

**Figure 6**

The number of structures deposited annually in the Protein Data Bank. Structures determined with the use of synchrotron radiation are represented in green, and those with conventional sources in red. The data represent the time window between 1985 and 2009, with statistics for the latter still incomplete. The figure was provided by Heping Zheng.

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