The start of a new generation: the present status of the SPring-8 synchrotron and its use in structural biology

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Structure August 1999, 7:R183–R187
http://biomednet.com/elecref/09692126007R0187

Introduction

Recently, the number of available three-dimensional structures of biological macromolecules has grown a pace, with as many as 2000 registrations to the Protein Data Bank in 1998. A large portion of these structures have been determined using synchrotron radiation. Most of these results have been obtained from second generation synchrotron radiation facilities: Cornell High Energy Synchrotron Source (CHESS, USA); Hamburger Synchrotronstrahlungslabor (HASYLAB, Germany); the National Synchrotron Light Source (NSLS, USA); the Photon Factory (PF, Japan); the Synchrotron Radiation Center (SRC, UK); and Stanford Synchrotron Radiation Laboratory (SSRL, USA).

The demand on synchrotron radiation beamtime for the study of structural biology and structure-based drug design is ever increasing. In the past few years, three third generation synchrotron radiation facilities which produce radiation in the hard X-ray region have come into operation: the European Synchrotron Radiation Facility (ESRF) at Grenoble, France; the Advanced Photon Source (APS) at Argonne, USA; and SPring-8 at Nishi-Harima, Japan. Studies of structural biology comprise one of the major projects to be carried out at these facilities and at SPring-8 several beamlines for use in such studies are currently in operation or under construction. We describe here an outline of the SPring-8 project, the available beamlines, scientific output and utilization of the facility in conjunction with macromolecular crystallography.

The advantages of third generation synchrotron radiation

The main feature of third generation synchrotron radiation facilities is the straight sections in their storage rings in which insertion devices, such as undulators and wigglers, can be installed. The radiation produced by these synchrotrons, especially X-rays from insertion devices, offer several advantages: tunability within a broad range of wavelengths, the small cross-section of the X-ray source, the small divergence of X-rays, and strong intensity. These properties make possible a range of X-ray diffraction experiments that are impossible with laboratory X-rays or difficult with the second-generation synchrotron radiation.

(1) Diffraction experiments from very small, thin crystals. Such experiments are important when only a small amount of sample is available. Moreover, the growth of large crystals requires much time, money and manpower, and obtaining large crystals from artificial proteins can be difficult.

(2) Diffraction experiments on crystals with very large unit cells and/or the determination of high-resolution structures. With the superior collimation and intensity of X-rays provided by third generation synchrotron radiation, it is much easier to collect good diffraction data. The small divergence from undulators provides much less background scattering, allowing good signal-to-noise ratios at high diffraction angles.

(3) Structure analysis using multiwavelength anomalous diffraction (MAD). Structure analysis using MAD techniques facilitates the rapid determination of unknown structures, avoiding the difficulties of isomorphism encountered by conventional isomorphous replacement methods. MAD techniques are only possible with synchrotron radiation and can provide electron-density maps within a very short period of time.

(4) Time-resolved experiments. The high flux of white X-rays from wigglers or unmonochromatized X-rays from undulators make it possible to carry out experiments in short time slices.

In practice, the availability of third generation synchrotron radiation accelerates structure determination. The high-resolution data obtained from these synchrotrons allows both the rapid and more accurate interpretation of structures. The large number of structures obtained by these methods will prove useful for protein engineering, the design of rational mutation studies, and structure-based drug design. Thus, the use of third generation synchrotron radiation opens a new era in structural biology providing new opportunities over and above second generation synchrotron radiation.

What is SPring-8?

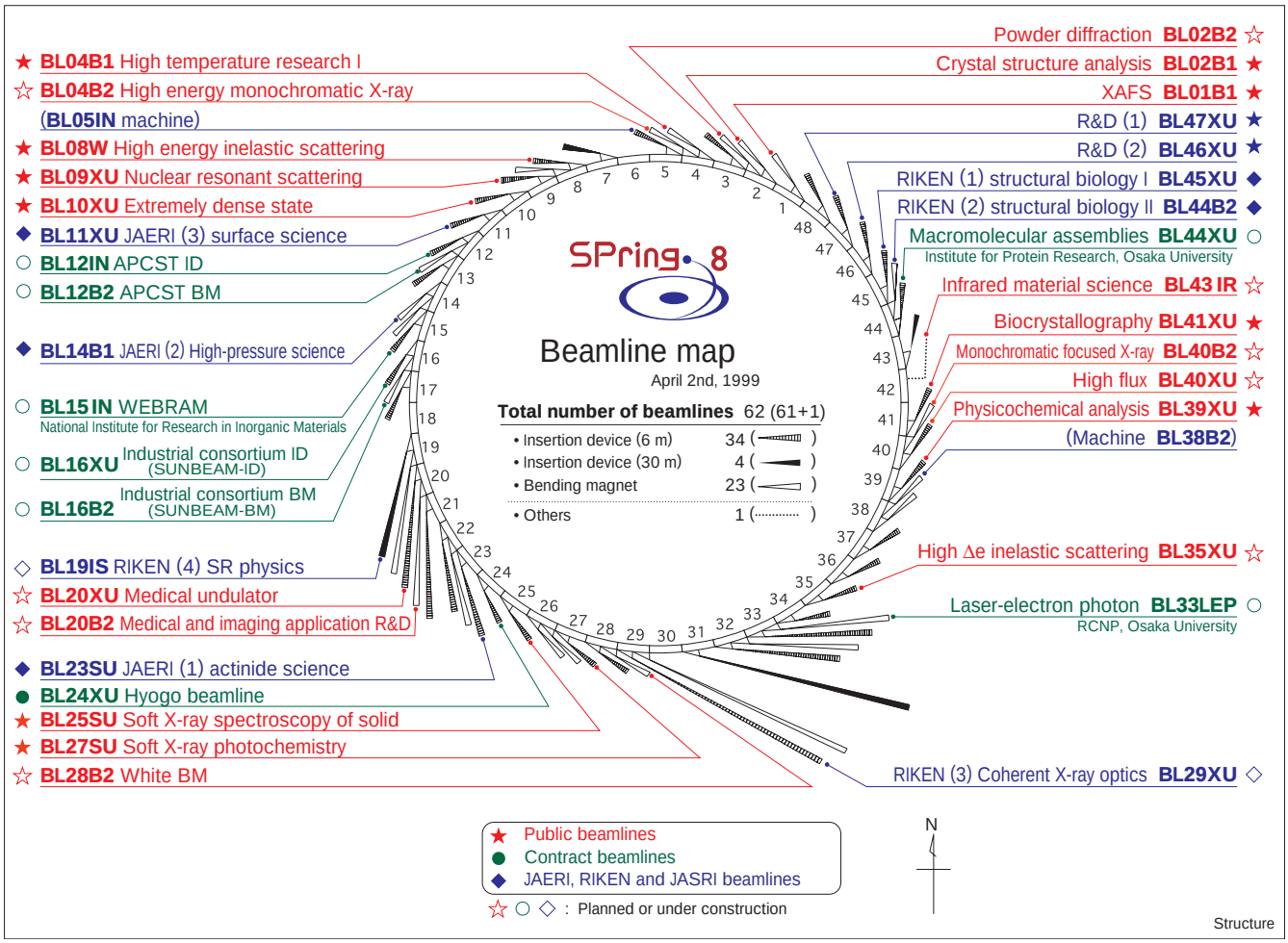
SPring-8 (*S*uper *P*hoton *ring* with *8* GeV) [1], located 100 km west of the Kobe City, is the worlds most powerful

third generation synchrotron radiation facility and is supported by the Science and Technology Agency of the Japanese Government. The accelerators of SPring-8 consist of an injector (a linear accelerator of 1 GeV and a booster synchrotron of 8 GeV) and a storage ring. The storage ring has an electron energy of 8 GeV and a stored current of 100 mA. The life-time of the electron beam in the storage ring is about 70–80 hours, and provides high-energy, high-flux, brilliant and parallel synchrotron radiation for a diverse range of research. The ring has 38 straight sections on which insertion devices can be installed to provide synchrotron radiation in the X-ray region from 500 eV to 300 keV. The accelerators and beamlines have been constructed in collaboration with JAERI (Japan Atomic Energy Research Institute) and RIKEN (Institute of Physical and Chemical Research). The facility was completed in October 1997 with ten beamlines for public use. The synchrotron has the potential for 62 beamlines to be constructed; at present 38 are either operational or under construction. These beamlines are illustrated in Figure 1. The operation of the accelerators and beamlines, management of the SPring-8 committees and technical assistance for researchers are all supported by the Japan Synchrotron Radiation Research Institute (JASRI). The beamlines at SPring-8 are roughly classified into two: beamlines for public use constructed using the governmental budget; and contract beamlines constructed by JAERI, RIKEN, universities, national research institutes or industries using their own budgets and for their exclusive use.

Characteristics of the beamlines for use in structural biology

The beamlines at SPring-8 for use in structural biology are listed in Table 1. With the exception of BL24XU, all of these beamlines are located in the life science zone, in the eastern part of the experimental hall of the storage ring, in order to make common use of various biochemical equipment.

Figure 1



The beamlines at SPring-8. Beamline BL38B1 is not shown in the figure as the construction plan for this beamline has not officially been approved.

Table 1

Beamlines for use in structural biology at SPring-8.

Beamline*	Experimental station	Institute	Status
Public beamlines			
BL38B1 (BM)	Crystallography, XAFS	–	Under construction
BL40B2 (BM)	Crystallography, small-angle scattering	–	Commissioning
BL40XU (U)	Time-resolved studies, small-angle scattering	–	Under construction
BL41XU (U)	Crystallography	–	Operational
Contract beamlines			
BL24XU (U)	Crystallography	Hyogo prefecture	Operational
BL44XU (U)	Crystallography	Osaka University	Commissioning
BL44B2 (BM)	Crystallography, XAFS	RIKEN	Operational
BL45XU (U)	Crystallography, small-angle scattering	RIKEN	Operational

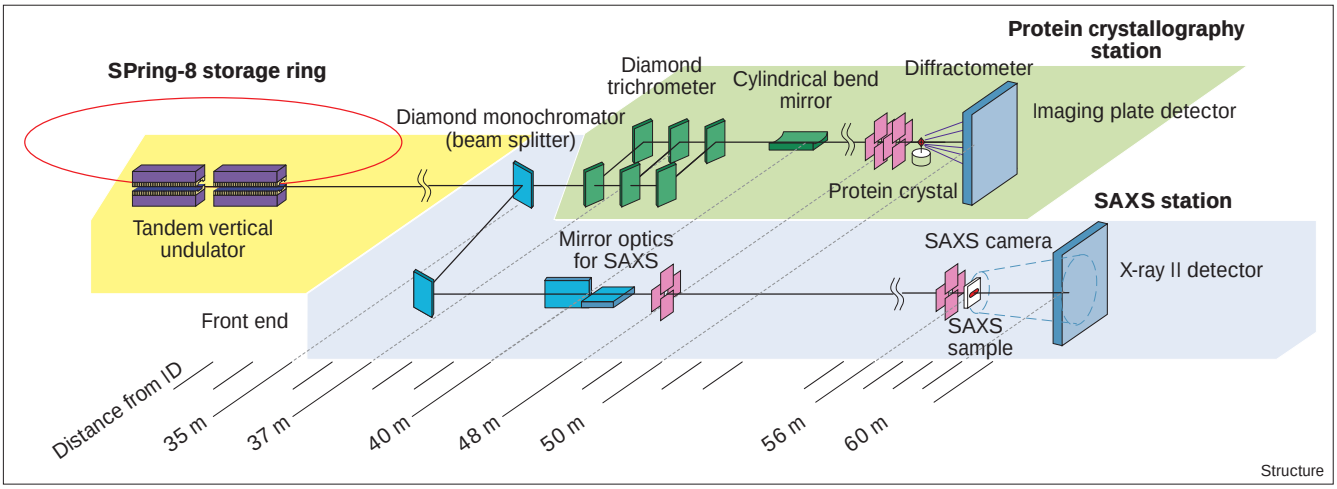
*BM, bending magnet beamline; U, undulator beamline.

All of the undulator beamlines have a focused beam that can be used to conduct diffraction experiments on thin crystals (e.g. ~10 μm thickness). These beamlines can also be used to perform low background scattering experiments, by utilizing a parallel undulator beam that matches the acceptance angle of the crystal. This feature also results in structure analysis at higher resolution than compared to experiments using laboratory X-rays or second generation synchrotron radiation.

At beamline BL41XU, the MIR-OAS method [2], which combines the heavy-atom multiple isomorphous replacement (MIR) method with high-energy X-rays and the optimized anomalous scattering effect (OAS), has been developed for the routine analysis of macromolecules. The high intensity of the beam expands the range of this technique in terms of molecular weight and crystal size.

Beamline BL45XU, constructed and operated by RIKEN, has a specific feature of interest [3,4]. The synchrotron radiation beam is split energetically by transparent diamond crystal monochromators so as to operate both crystallography and small-angle scattering experimental stations simultaneously, not in a time sharing mode. In addition, the crystallography beamline provides X-rays of three different energies to the crystals, facilitating the use of MAD techniques [5,6]. An outline of BL45XU is shown in Figure 2. The three X-ray beams with different energies

Figure 2



Outline of the RIKEN beamline I (BL45XU) for use in structural biology studies [4]. The main features of the beamline are illustrated; two tandem undulators and four sets of diamond crystal monochromators can be seen.

Table 2

MAD structure analyses at beamline BL45XU.

Sample	Anomalous atoms /asymmetric unit	Molecular weight /asymmetric unit	Status
1	1 Zn	23,000 × 1	Model refinement
2	4 Zn	13,000 × 4	Model refinement
3	3 Se	18,000 × 1	Model refinement
4	4 Hg	33,000 × 2	Model refinement
5	4 Zn	55,000 × 2	Model refinement
6	16 Hg	19,000 × 4	Model refinement
7	2 Zn	30,000 × 2	Model building
8	2 Zn	18,000 × 2	Model building
9	24 Se	50,000 × 2	Model building
10	4 Pt	55,000 × 2	Phase improvement
11	22 Se	75,000 × 2	Phase improvement

give diffraction patterns above and below the absorption edge of metal ions in the biological macromolecule, without the need to change beam alignment and within a very short time span. This beamline was designed to provide diffraction data with the high accuracy required for MAD techniques.

The BL44XU beamline was specially designed for the crystallography of macromolecular assemblies, such as viruses, using a parallel undulator beam. The high-flux beamline BL40XU provides a flux of more than 10^{14} photons/second from a helical undulator without the insertion of monochromators in its optics, and can be used for time-resolved experiments with low-energy resolution.

The bending magnet beamlines all contain similar optics, diffractometers, detectors and data acquisition systems to collect diffraction data in routine macromolecular crystallography; these features are also very similar to those of laboratory systems in order to enable researchers to carry out experiments with synchrotron radiation easily, as in their home laboratories. Beamline BL44B2 also utilizes white radiation from the bending magnet to carry out time-resolved crystallography. In contrast, beamline BL40B2 enables scattering images to be recorded from noncrystalline biological materials, using an X-ray beam with tuneable energy over a broad range.

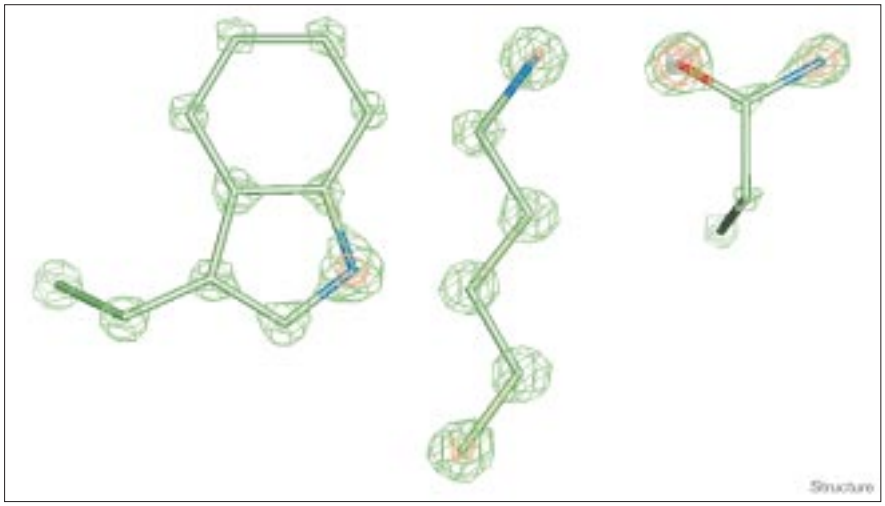
At present, conventional image plates are used as detectors. However, research and development programs have been driven towards speeding up the read-out time to 60 images per hour, and the first system capable of this detection rate will be available this winter. Charge-coupled device (CCD) detectors have also been introduced in some of the beamlines. Such high-rate detectors accelerate the data acquisition time, in some cases allowing a complete set of diffraction patterns to be collected in less than one hour.

Almost all experiments are carried out at liquid nitrogen temperatures in order to avoid serious radiation damage. Even at these low temperatures, however, damage has been observed in many crystals when the storage ring was operated at the stored current of 100 mA. Some scientists claim that experiments at even lower temperatures may protect crystals in future studies.

Utilization and scientific achievements

In October 1997, SPring-8 initiated its first phase utilization of the beamlines. Since then, beamlines BL41XU and BL45XU have been in operation for more than one and a half years, beamlines BL24XU and BL44B2 have been in operation for one year and beamlines BL40B2 and BL44XU will be in full service this fall.

Figure 3



Electron-density maps for three sidechains selected from the crystal structure of bullfrog egg lectin. The structure was refined at 1.06 Å resolution using diffraction data measured at the RIKEN beamline BL44B2. (The figure was reproduced with courtesy of T Nonaka.)

Of all these beamlines, perhaps some of the most remarkable results have been obtained from BL45XU, reflecting the powerful use of the MAD technique for structure determination. More than ten structures have been solved using BL45XU in conjunction with the MAD technique, and additional experiments have also been performed using this method. A list of structure analyses performed using this beamline is given in Table 2. As can be seen in the table, the anomalous scatterers are either the selenium atom in selenomethionine or other heavy atoms used for the preparation of derivatives in the isomorphous replacement method (MY and T Kumasaka, unpublished results). All of these analyses will be published in the near future. The results of studies carried out using beamline BL44B2 provide a further example of the exceptional atomic resolution structure analysis possible at SPring-8. Figure 3 shows the electron-density maps for the sidechains of three amino acid residues in the crystal structure of bullfrog egg lectin refined at 1.06 Å resolution (T Nonaka, T Matsumoto and Y Mitsui, unpublished results), illustrating the high resolutions attainable with this facility. Further experiments are continuing to obtain even higher resolution data, and other crystals have been analyzed to similar levels (e.g. using a wavelength of 0.7 Å, diffraction patterns of up to 0.84 Å resolution were recorded from a crystal of a flavin mononucleotide binding protein).

How to access SPring-8

At SPring-8, public beamlines are open to all scientists and engineers, irrespective of domestic or overseas proposal. There are three modes of utilization: regular experiment; test experiment (to test crystallinity and cryoconditions); and urgent experiment. There are also categories of 'proprietary/non-proprietary' use. SPring-8 does not charge for non-proprietary use of the beam, if the results are open to the public (i.e., published in journals). SPring-8 starts proprietary use for the period 1999B (i.e. the later half of the year) in September 1999.

In three periods of utilization of the public beamline BL41XU, 1997B, 1998A and 1999A, the number of proposals accepted were 22(36), 39(60) and 59(73), respectively, where the number in parentheses was the number proposed. At the end of June this year, BL41XU was the only operational public beamline and beamtime was therefore very limited. However, the completion of two additional public beamlines, BL38B1 and BL40B2, is expected to alleviate this problem.

For regular and test experiments, applications for beamtime at SPring-8 are called twice a year, following the procedure outlined on the internet Web site. Proposals are reviewed by the SPring-8 Proposal Review Committee (PRC). Accepted proposals are valid for the utilization period (e.g. in the case of 1999B, the utilization period is limited to three months from September to December) and beamtime will be allocated within this period. For urgent experiments, applications for beamtime can be submitted and reviewed at any time. The SPring-8 steering committee decided that a portion of the beamtime of contract beamlines should be given to JASRI for their use. The same portion of beamtime in the RIKEN and JAERI beamlines is also to be given to JASRI for public use.

For further detailed information on SPring-8, refer to the SPring-8 home page at the internet web site (http://www.spring8.or.jp/).

Acknowledgements

We would like to acknowledge N Kamiya, S Adachi, T Kumasaka, T Nonaka and Y Morimoto for supplying valuable information and data to write this article. We also wish to thank all members of the SPring-8 project who supported beamline construction and beamline commissioning.

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