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Trichromatic Concept at SPring-8 RIKEN Beamline I

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SPring-8 RIKEN beamline I has been designed and developed for structural biology research by the Institute of Physical and Chemical Research (RIKEN). The beamline consists of two experimental stations for protein crystallography and small-angle X-ray scattering. Both types of experiments can be carried out simultaneously, with dichromatic synchrotron radiation emitted from two coaxial undulators with vertical polarization. The branched beams are generated by a transparent diamond crystal. With synchrotron radiation, the multiple-wavelength anomalous-dispersion (MAD) method, which gives phases from a single anomalous scatterer, has been developed. Anomalous scattering contributes a small proportion of the diffraction intensity so that the accuracy of intensity data is important. The protein crystallography branch of RIKEN beamline I has been designed based on a 'trichromatic concept' to optimize MAD data collection. This concept requires the quasi-simultaneous collection, by use of a 'trichromator', of three intensity data sets at three different wavelengths from a single protein crystal without changing any settings. The main feature of the concept is the minimization of systematic errors in the measurement of anomalous diffraction for the MAD method. Initial commissioning of the beamline has provided three different monochromated undulator beams, which were successfully observed on the phosphor screen located at the near end of the trichromator.

Keywords: macromolecular crystallography; MAD method; trichromatic concept; trichromators.

1. Introduction

Proteins consist of 20 types of amino acids, and many have been studied based on knowledge of the primary sequences. However, the functions of proteins are not yet understood in terms of their three-dimensional structures. Structural knowledge of biological macromolecules at atomic resolution is essential for understanding their functions. The accumulation of three-dimensional structures plays an important role in protein engineering and drug design.

The first three-dimensional structure of a protein, myoglobin, was determined by X-ray crystal structure analysis methods in 1958 (Kendrew *et al.*, 1958). Since then, X-ray crystallography has become the most important tool for the determination of three-dimensional structures of biological macromolecules. Structures of a wide variety of biological macromolecules have been determined at atomic resolution and many biological function studies have been made based on the structures determined by X-ray crystallography.

The introduction of synchrotron radiation has accelerated the accumulation of three-dimensional structures. Synchrotron radiation has many advantages over conventional laboratory X-ray sources, *i.e.* high flux at the sample position, a highly collimated beam and tunability over a wide energy range, and the availability of high-energy X-rays. For instance, high flux at the sample position reduces the exposure time of a weakly diffracting crystal and a highly collimated beam improves the signal-to-background ratio. Therefore, synchrotron radiation is a most important and indispensable tool for macromolecular crystallography (Helliwell, 1992).

With the tunability over a wide energy range of synchrotron radiation, the multiple-wavelength anomalous-dispersion (MAD) method, which gives phases from a single anomalous scatterer (Karle, 1980), has been developed (Hendrickson *et al.*, 1988; Hendrickson, 1991). Utilizing third-generation synchrotron radiation and with the maximized advantage of the MAD method, RIKEN beamline I has been designed for structural biology research by the Institute of Physical and Chemical Research (RIKEN).

2. Multiple-wavelength anomalous-dispersion method and the trichromatic concept

The most critical problem in macromolecular crystallography is the phase problem. The multiple isomorphous replacement (MIR) method is the most powerful phasing method in macromolecular crystallography. However, the method requires at least two different heavy-atom deri-

vatives, the crystals of which have to have a high degree of isomorphism with the native crystal.

On the other hand, with MAD methods all the data collection can be made on a crystal that contains an anomalous scatterer, and isomorphism is perfect. The MAD method has the great advantage of accuracy and convenience in phase evaluations.

The essential requirement for the MAD method is tunability of the wavelength; high flux and high-energy resolution are also important. These requirements are satisfied by third-generation synchrotron radiation facilities. However, the development of the MAD method as a routine macromolecular crystallographic method is not straightforward because the contribution of anomalous diffraction is minimal.

To ensure the accuracy of MAD experiments, it is essential to minimize systematic errors, such as those from absorption, detector characteristics and radiation damage, so that the actual signals in Bijvoet and dispersive differences are as precisely measured as possible. In MAD experiments, at least three sets of diffraction data have to be collected at three different wavelengths. The wavelengths also have to be tuned as quickly as possible with good reproducibility. To realize such an experimental environment, the 'trichromatic' concept has been introduced by developing high-quality diamond crystals. The main feature of this concept is the minimization of systematic errors during anomalous-scattering measurements for the MAD method.

The trichromatic concept requires that three sets of diffraction data at three wavelengths are collected sequentially, by use of a 'trichromator', within a short period of time from an identical crystal for each data set and without changing any settings. The basic concept of the experiment is summarized in Fig. 1.

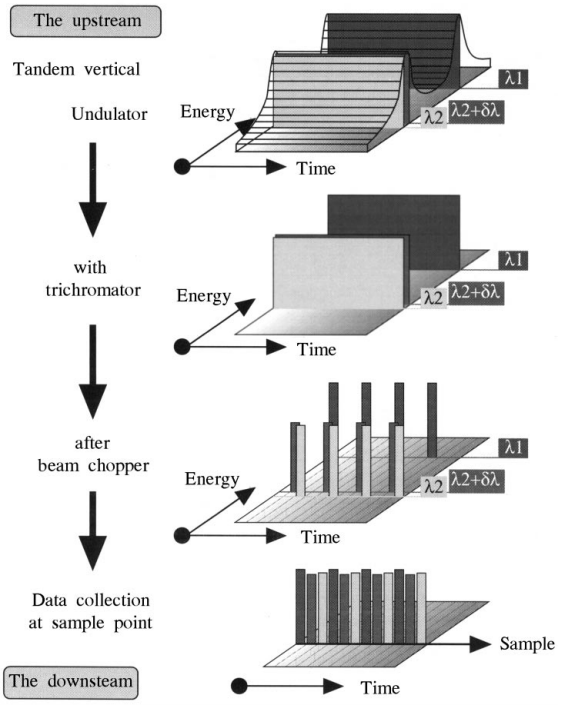

Figure 1
Summary of the 'trichromatic concept' (see description in the text).

Dichromatic undulator beams, emitted from tandem vertical undulators, are introduced into the trichromator, which consists of three pairs of double-crystal monochromators. The crystals are transparent high-quality diamonds. The trichromator produces three different monochromated undulator beams (λ_1, λ_2 and $\lambda_2 + \delta\lambda$) from a dichromatic undulator beam. For MAD data collection with a trichromator, the first wavelength (λ_1) is set at a remote point of absorption edge for an anomalous-scattering-free data set. The other two wavelengths (λ_2 and $\lambda_2 + \delta\lambda$) are extracted near and on the absorption edge to maximize the anomalous-scattering contributions. The three beams are initially tuned at three wavelengths for each data collection and are sequentially supplied without change of setting, except for the beam choppers, which select one wavelength from the trichromatic undulator beam. Data collection is sequentially performed at each wavelength to minimize the background contributions from the other wavelengths.

3. Beamline design

RIKEN beamline I has three notable features. The first is the trichromatic concept for MAD experiments, which is a multi-color application of synchrotron radiation. The

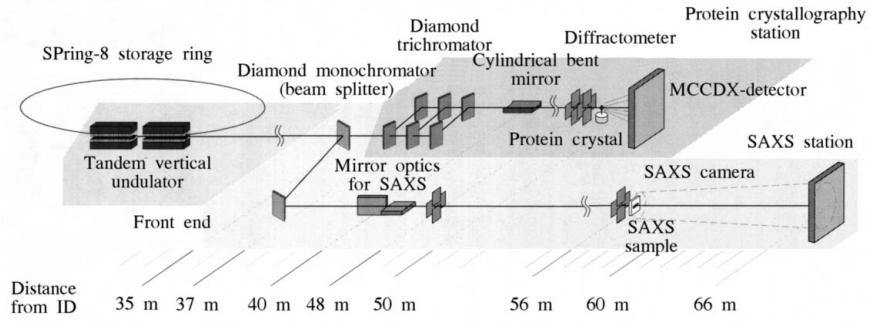

Figure 2
'Bird's eye' view of RIKEN beamline I for structural biology.

second is tandem vertical undulators optimized for horizontal branching and multi-color application. The third is beamline branching with a diamond monochromator, which allows simultaneous operation of the small-angle X-ray scattering and protein crystallography experimental stations.

The synchrotron radiation beam is provided so as to allow the two stations to be operated simultaneously. A schematic outline of the beamline is shown in Fig. 2. In order to provide beams of different energies to the two experimental stations including the trichromatic concept, two undulators are arranged serially as a tandem vertical undulator. The undulators are of identical in-vacuum design, vertically polarized as a result of horizontal branching. Each undulator has 37-pole permanent magnets with a period length of 37 mm, and emits a beam with photon energies from 6 to 14 keV as a fundamental harmonic (Tanaka *et al.*, 1998). The calculated spectra for the vertical undulator are shown in Fig. 3.

Dichromatic synchrotron radiation emitted from the two undulators is branched by a beam splitter, a transparent diamond monochromator (Yamaoka *et al.*, 1995). The beam-splitter is positioned at 34 m from the light source and acts as a first monochromator for the SAXS branch beamline. The beam, which goes through the beam splitter, is guided to the trichromator for protein crystallography.

The trichromator, which consists of three pairs of transparent diamond monochromators with fixed exits, collinearly introduces three monochromated undulator beams in identical beam directions. The trichromator is positioned 37 m from the light source. It uses synthetic diamonds (Sumitomo Electric Industries Co. Ltd) (Uruga *et al.*, 1995) with sizes ranging from 5 × 4 to 7 × 5 mm. The reflection planes of the diamonds are chosen to be (4 0 0) with Bragg geometry, and the fundamental energy range covered is from 7 to 16 keV. As the glancing angles to the diamond crystals are close to 45° in this energy region, the polarization of the beam has to be vertical to obtain a certain level of intensity.

The mechanisms of the trichromator are shown in Fig. 4. The first and second crystals are mounted on four- and three-axis goniometers, respectively. The four-axis goniometer for the first crystal of each pair consists of Bragg-angle-rotation and fine-rotation stages ($\theta1$ and $d\theta1$), a translation stage (dL) along the direct-beam direction, an x-translation stage ($dx1$) on the rotation stages, and crystal-tilting and fine-tilting stages ($\varphi1$ and $d\varphi1$). All three translation stages of the first crystal are directly driven by linear motors, and move on a common linear translation guide. The fine-rotation and fine-tilting stages are driven by piezo-translators with elastic bending stages. The three-axis goniometer for the second crystal has a Bragg-angle rotation stage ($\theta2$) with a high-resolution encoder. A beam chopper is situated between the first and second crystals. The beam chopper sequentially supplies one wavelength out of three to the experimental station. Construction of the trichromator was completed at Sigma Koki Co. Ltd, Japan.

A cylindrical bent mirror, located at 39 m from the light source, removes higher order harmonics and collimates the undulator beam.

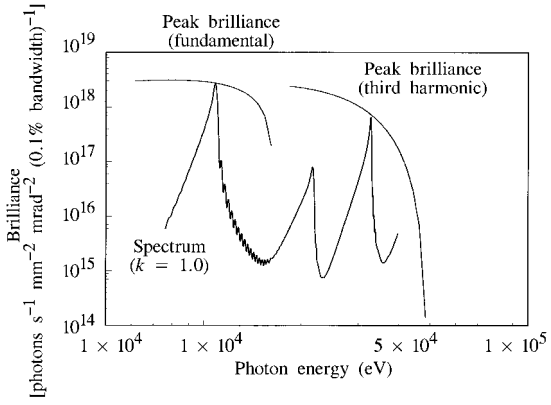

Figure 3
Spectra of the vertical undulator. These data were calculated using the *Synchrotron Radiation Calculation* program (H. Kitamura, personal communication).

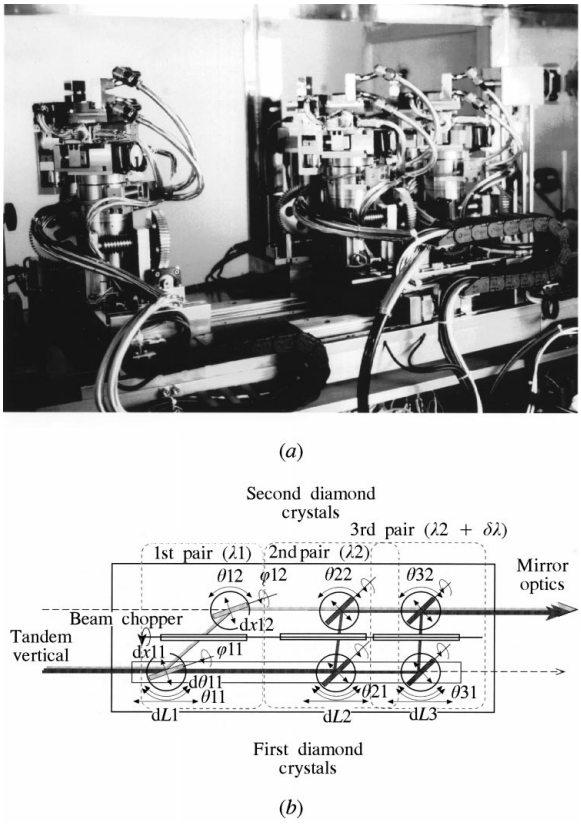

Figure 4
(*a*) Side-view photograph of the trichromator and (*b*) the configuration of the trichromator mechanism. The trichromator contains three pairs of diamond double-crystal monochromators. The first and second crystals are mounted on four- and three-axis goniometers, respectively.

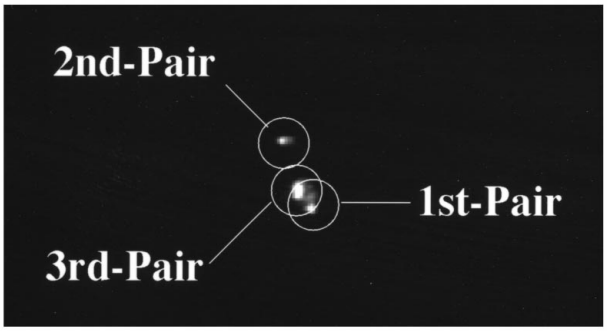

Figure 5
The first image of three undulator spots with three different wavelengths.

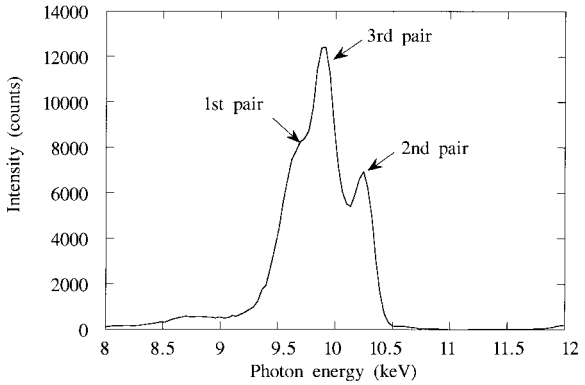

Figure 6
Spectrum of the trichromatic undulator beam from the trichromator.

In the experimental station, sample crystals are aligned by a four-circle goniometer, located at 50 m from the light source.

The detector is the other important device in MAD experiments. A fast-readout two-dimensional detector is most suitable for high-precision MAD experiments. A multiple CCD X-ray detector (MCCDX) has been developed to record the diffraction patterns of protein crystals (Kumasaka *et al.*, 1996). The MCCDX is a 4 × 4 matrix array of a CCD X-ray detector module with an active area of 200 × 200 mm. Each module of the CCD X-ray detector consists of a scintillation screen, a fiber-optic taper and a large-format scientific CCD with 1k × 1k pixels. The area reduction ratio of the fiber-optic taper is 25%. The CCDs are operated at 273 K. Construction of the MCCDX was completed at EEV, UK.

4. Observation of the trichromatic undulator beam

The construction of RIKEN beamline I started at the SPring-8 site in August 1996 and the commissioning of the beamline with synchrotron radiation commenced in July 1997.

The initial test of the trichromator was performed using diamond crystals of thickness 1 mm and a storage ring current of 1.0 mA. The trichromator successfully monochromated three wavelengths at the same time, and three undulator beams were observed as three spots on the phosphor screen (Fig. 5). In Fig. 5, the spots are displaced relative to one another to give spots at different energies. The three spots were independently controlled by each of the pairs of diamond monochromators. Then the energies of the first, second and third pairs of the trichromator were tuned at 9.7, 9.9 and 10.2 keV, respectively. Each diamond crystal of thickness 1 mm transmits almost 30% of the incident beam in this energy region. The energies of these three spots were detected by measuring the scattering of X-rays by air. The spectrum of the trichromatic undulator beam, which was measured using an Si-SSD detector, is shown Fig. 6.

5. Summary

The protein crystallography station at RIKEN beamline I has been designed based on a 'trichromatic concept' optimized for MAD data collection. The initial construction phase has been completed and the beamline is currently being commissioned with synchrotron radiation. As the first step of commissioning, the trichromator successfully monochromated three wavelengths at the same time.

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