6 Structural Biology SAKABE Project

6-1 A Fully Automatic High-speed Weissenberg Data Collection System

The "Galaxy" data collection system consists of automatic data collection part (ADCP), installed at BL-6C, and data processing and storage systems [1] mainly installed in the Structural Biology SAKABE project (SBSP) house, located 30 m from the PF ring. The two parts are connected with a high-security, high-speed network. Fig. 1 shows the ADCP. The X-ray beam comes from the left side of the figure. The monochromator housing, camera, IP reader, IP eraser and IP cassette transportation mechanism are arranged sequentially. In order to obtain X-rays as intense as possible from the 2.5 GeV bending magnet beamline, we have installed a rotated-inclined focusing monochromator [2] which can cover the 0.8-2.0 Å wavelength range without changing the monochromator crystal. The first bench as shown in Fig. 1 is introduced to choose the camera position according to the wavelength of the primary X-rays. Both the IP reader and eraser are always fixed to 2θ equal to zero. The second 2θ arm is used to transport the IP cassette between the camera, IP reader and eraser positions. The Galaxy system installed at BL-6C is shown in Fig. 2, where the camera is set at a wavelength of 0.9 Å and two IP cassettes are set at the camera and IP reader positions.

One of the special characteristics of this system is a Weissenberg camera with a pair of movable cylindrical

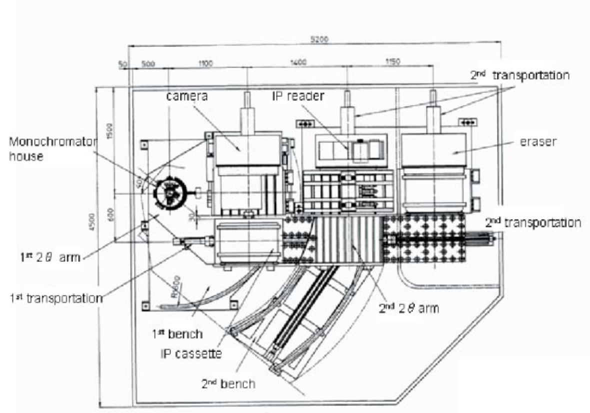

Figure 1
Schematic drawing of Galaxy at BL-6C.

Figure 2
Galaxy installed at BL-6C. The chairman of the JSPS-169 committee; Honorary Professor of KEK Noriyoshi SAKABE who designed Galaxy, is standing at the left side of the photograph.

Table 1 Characterization of Galaxy.

classification	Items	Contents
IP-cassette	Type Radius and width Windows to take incident X-ray Maximum 4θ angle Number of frames/ cassette (for 1.9 Å, with 1 Å X-rays) Frame exchange speed Translation for Weissenberg mode Speed of IP-cassettes transportation	Fully cylindrical and movable 400 mm, 430 mm Every 10 deg 180 deg 12 for asymmetric setting 6 for symmetric setting 8.6 deg/sec Maximum 10 mm 4 min for cassette exchange
IP-reader	Number of reading heads Reading mode Range Reading time	Parallel 5 IP-cassette rotation (3 turns/sec) 2~3×10^6 X-ray photons 9 min with 0.1×0.1 mm^2 pixel size
IP-eraser	Number of fluorescent lamps & sodium lamps Erasing mode Erasing time	30 W × 8, 160 W × 8 IP-cassettes rotation 30 sec
X-ray generator	Use to make the correction table among the relative sensitivities among 5 reading heads on IP-reader	30 kV, 1 mA

IP cassettes. The overall characteristics of the data collection system are summarized in Table 1. The cylindrical IP cassette, a specially ordered IP of 450 mm × 2,513 mm size from Fuji Photo Film Company Ltd. has 36 small rectangular holes (3 mm×15 mm) at equal intervals along the circumference of the cylinder every 10° to allow the primary X-ray beam to hit the sample crystal. There are two available selections of exposure area, a symmetric setting (S) and an asymmetric setting (A). With the exposure area chosen as either 60° for the symmetric setting or 30° for the asymmetry setting, 6 or 12 frames can be recorded on one IP cassette, respectively. In both cases, the resolution limits along the equatorial and horizontal axes are 1.93 Å and 2 Å for 1 Å X-rays. Two IP cassettes are employed in this system in order to speed up the data collection. While the data on one cassette are being read and erased, another cassette is used for recording.

A cylindrical helium chamber with a beryllium window is set at the center of the camera shown in Fig. 2. The sample crystal, a beam stop, a telescope to observe the sample and a cryo-cooling head are positioned in the helium chamber, and a computer-controlled goniometer is used for centering the sample with a semi-automatic crystal alignment system.

The high-speed IP reader is equipped with five sets of reading heads aligned at intervals of 900 mm. Two 16-bit ADCs are used for reading each head to extend the range of the IP reader to 1×10^{6} X-ray photons. Background noise is 3%, and is measured after uniform exposure at a level of 8,600 X-ray photons.

Galaxy can be operated by selection from a menu coded by a GUI on a computer screen. In order to perform data collection for time-resolved crystallography, a special menu is provided. When the Weissenberg mode for the data collection is selected, the axis to be aligned can be selected from a list.

For automatic data collection, a high speed network system connected with 1000BaseSX and a data server with a large disk space (300 GB) and high-speed memory are provided, and there are three computers situated around BL-6C in the PF experimental hall and computer systems for data processing in the SBSP house.

References

[1] N. Sakabe, K. Sakabe, T. Higashi, N. Igarashi, M. Suzuki, N. Watanabe and K. Sasaki, *Nucl. Instr. and Meth. A* **467-468** (2001) 1367.

[2] N. Watanabe, M. Suzuki, Y. Higashi and N. Sakabe, *J. Synchrotron Rad.,* **6** (1999) 64.

6-2 Observation of d-Electron Redistribution from Zinc Ions in 2Z Insulin

As an example of the use of Galaxy, we show here a world record result. The Sakabe group (consisting of N. Sakabe, K. Sakabe and K. Sasaki) has used the system to obtain 0.8 Å resolution data of recombinant human 2Zn insulin crystals under cryo conditions, and also succeeded in observing the d-electron redistribution in zinc ions by perturbation due to the three ligating nitrogen atoms of imidazole and three water molecules (Fig. 3) [1, 2]. This study is still in progress, and the results will be published elsewhere. Similar observations have already been made by the same group using 1.2 Å resolution data from pig 2Zn insulin crystal collected with Ni filtered CuKα radiation on a Hilger & Watts four-circle diffractometer placed in a room maintained at 4°C [3].

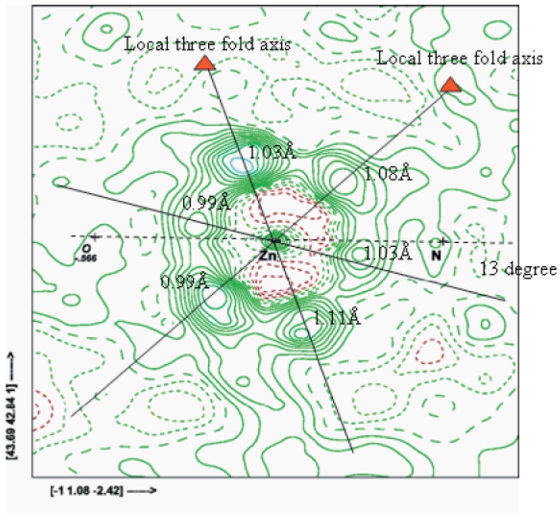

Figure 3
A section of difference Fourier map showing four corners of the cube of the octahedral arrangement by ligands and the zinc ion. The directions of the peaks which are estimated to be due to the d-electron redistribution in the zinc ion fit nicely to the corners of the cube (local three fold axis). Their distances from the zinc ion became shorter than previous results by about 0.3 Å, but this is still longer than the expected value. This can be expected due to the limited resolution of the map.

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References

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