Conceptual design of novel IP-conveyor-belt Weissenberg mode data collection system with multi readers for macromolecular crystallography. A comparison between Galaxy and Super Galaxy

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Galaxy is a Weissenberg type, high speed, high-resolution and highly accurate fully automatic data collection system using two cylindrical IP-cassettes each with a radius of 400mm and a width of 450mm. It was originally developed for static three-dimensional analysis using X-ray diffraction and was installed on bending magnet beamline BL6C at the Photon Factory. It was found, however, that Galaxy was also very useful for time-resolved protein crystallography on a time scale of minutes. This has prompted us to design a new IP-conveyor-belt Weissenberg-mode data collection system called Super Galaxy for time-resolved crystallography with improved time and crystallographic resolution over that achievable with Galaxy. Super Galaxy was designed with a half-cylinder shaped cassette with a radius of 420mm and a width of 690mm. Using 1.0Å incident X-rays, these dimensions correspond to a maximum resolutions of 0.71Å in the vertical direction and 1.58Å in the horizontal. Upper and lower screens can be used to set the frame size of the recorded image. This function is useful not only to reduce the frame exchange time but also to save disk space on the data server. The use of an IP-conveyor-belt and many IP-readers make Super Galaxy well suited for time resolved, monochromatic X-ray crystallography at a very intense third generation SR beamline. Here we describe Galaxy and a conceptual design for Super Galaxy and compare their suitability for use as data collection systems for macromolecular time-resolved monochromatic X-ray crystallography.

1. Introduction

It has long been believed that X-ray crystal analysis is useful only for static structure analysis and that it has no power to solve dynamic structures. This belief has been changed, however, with the advent and development of synchrotron radiation X-ray crystallography. The group of K. Moffat has successfully collected a Laue image from a crystal of myoglobin in just 50psec using the wiggler beamline ID09 at the European Synchrotron Radiation Facility (ESRF). By this method, nanosecond time-resolved crystallography using laser photolysis of a carbon monoxide complex of myoglobin was achieved (Srajer et.al. 1996). They also showed that the quality of a single-pulse Laue diffraction pattern from cutinase was sufficient to refine the excited state structure in a reaction pathway from a known ground state (Wulff et.al. 1997).

The authors also started to develop the time-resolved Laue method at the PF in 1988 and constructed a prototype time-resolved Laue camera. A large part of this work was begun in 1993 supported by Grant-in aid for Scientific Research on Priority Areas from the Ministry of Education. A camera incorporating streak type time-resolved Laue and Weissenberg capability (Sakabe et. al., 1995) was installed at BL18B (Watanabe et.al. 1994). In the course of the study, we found that it is very difficult to develop a generalized time-resolved Laue method for studying reaction mechanisms because of the large mosaicity of protein crystals, the inability to find a suitable trigger and most of protein reactions being irreversible. It did become apparent, however, that many enzyme reaction rates in crystals are on the order of seconds to minutes. With this in mind and considering that most of protein structure analysis is carried out using monochromatic radiation, it was deduced that monochromatic time-resolved X-ray analysis would be superior to the Laue method for the study of enzyme reactions if a reasonably fast data collection system could be developed.

Galaxy (Sakabe 2001), which was developed and installed at station BL-6C at the PF, was a candidate instrument for the successful collection of time-resolved crystallographic data using monochromatic X-rays. The frame exchange rate (8.6deg/sec) was slower than expected, however, due to the inertia of the heavy IP-cassette and the resolution was severely limited by the number of frames that could be accommodated on a single cassette. It was necessary to collect at least two complete data sets on a single cassette because the time to change cassettes was prohibitively long (4min) for most enzyme reactions and even if a second cassette were used, the data were not well matched to those collected in the first stage of the reaction.

Thus, we have designed a new data collection system called Super Galaxy for time resolved monochromatic X-ray crystallography. This system would facilitate time-resolved structural analysis with improved time and crystallographic resolution and would be well suited to a high-brilliance third generation beamline.

2. Basic concept of the design of the instrument

2.1. Why two data sets per crystal?

The start of a reaction can be controlled to some degree by the trigger. However, once the reaction starts, the reaction phase of each molecule in the crystal cannot be synchronized. That is, at any given instant, molecules will exist in a range of intermediate state along the reaction pathway. Difference Fourier maps between two data sets collected at different stages of the reaction are useful to detect the change in structure. There are three different ways in which a reaction can be stopped. The first is that it is freeze-trap method. The advantage of this method is that it is possible to stop the reaction at short time intervals. It is rather difficult, however, to be confident about small differences in electron density and/or temperature factors because the data derive from different crystals removed from the reaction mixture at different time. The second approach is to use the freeze-trap method on a single crystal, namely, the (freeze-trap)-(melting-reaction) method. This method is rather complicated and it is difficult to estimate systematic errors inherent to the approach. The third method does not attempt to trap the intermediate states. Rather, two or more data sets are collected successively from one crystal during the reaction. This method minimizes any difference in experimental conditions between the data to be compared and is, therefore, superior to the above two methods when investigating small differences in electron density and temperature factors. It is expected that the existence of an intermediate structure will show up as an area of disorder. For example, in the case of a rhombohedral 2Zn insulin crystal, rotation of some of the $O\gamma$ atoms of A12Ser$O\gamma$ by 180deg around the $C\alpha - C\beta$ axis is expected to show up as disorder. As described above, the D-Fourier maps are usually used to study intermediate structures. Simulation calculations were performed to study the effect of random errors in the structure factor on the peak height ratio between the disordered atom position and

the most intense ghost peak in the D-Fourier map. In these simulations the peak intensity of the disordered atom was calculated as a number of electrons which gave the occupancy. These results are shown in Fig.1. In a conventional freeze-trap experiment, the random error between sets of structure factors obtained from two different crystals will be of the order of 10~15% and the R-factor between Fo and Fc will be in the range of 12~18%. If we use as a selection criterion the condition that the peak height ratio (PR) between the disordered atoms and the most intense ghost peak is 2.0, an R-factor of 10~15% gives 2.0~3.12 electrons at the disordered position, ie, an occupancy of 0.25~0.39 O atoms is needed to detect the disorder. In this method, however, where multiple sets of Fo are collected from a single crystal and all other experimental conditions are the same, the R-factor can be reduced to 1% and as a result, as few as 0.21 electrons can be detected at the disordered position – 10~15 times less than is possible with the freeze-trap method.

However, in this case the time required to collect a complete data set must be short in compare to the rate of structure change. Thus strong X-ray source is need for this method
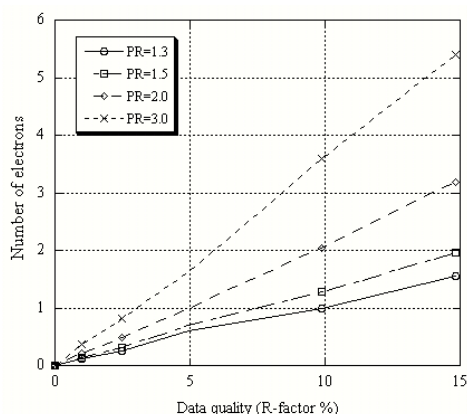
Figure 1

The relation between data quality and number of electrons of a target atom versus Peak Intensity Ratio (PR) of the target atom and the highest ghost in D-Fourier map. The D-Fourier map as a coefficient of [Ft(h)-Fo(h)] is calculated using rhombohedral 2Zn insulin data at 1.5A resolution where Fo(h) is observed structure factors and Ft(h)=Ft'(h)[1+k(rand-0.5), Ft'(h)=Fo(h)exp(-iα)+f[-k'exp(2πihr)+ k'exp(2πihr')], α is phase of Fo, f is atomic scattering factor of oxygen including the correction for the temperature factor effect, r and r' are the coordinates of rhombohedral 2Zn insulin A12SerOγ and it's disordered atom which are calculated by 180°rotation around Cα- Cβ axis of the A12Ser, respectively, and k' is the occupancy of the disordered atom which is related to number of electrons and rand is random number (0.0~1.0) calculated by the program rand, and k=0, 0.04, 0.1, 0.2, 0.4, 0.6 which values closely correspond to R-factor between Ft and Fo of 0, 1, 3, 5, 10, 15%, respectively.

2.2. Why an imaging plate (IP) ?

The time resolution of this study is determined by the time required to record a complete data set. Clearly, this time should be as short as possible so as to obtain a well defined result. In the selection of a detector system for time-resolved, high-resolution macromolecular crystallography, high sensitivity, large dynamic range and large active area are the most important criteria. While the read out speed of a CCD detector is of the order of 1~2 sec per frame, something like 100 frames need to be recorded in order to complete a data set using oscillation method. In fact, very often, due to the limited dynamic range of the CCD (10^4) more frames are required. As a result, the CCD spends more than 2min on data read out alone before any consideration is given to exposure time. IPs are one of the most powerful detector systems for collecting diffraction data because they have a large detection area (450mm wide by more than 20m in length), high sensitivity, large dynamic range (~10^6 order), high accuracy, reasonable pixel size and flexibility in shape. The major advantage of large format IPs for those studies is that several time-dependent data sets can be recorded on the IP and subsequently read out. Thus, the time resolution is the time of frame exchange plus the exposure time only and it is not necessary to include the read out and erasure time. This is the case for both Galaxy and Super Galaxy. Due to the large physical area of the IP, a complete set of time-dependent data image can be stored on a single IP cassette of Galaxy, especially when Weissenberg techniques are applied. Super Galaxy can store even more images on the IP-conveyor-belt before reading.

Due to their physical flexibility, the IPs can be fit readily to a cylindrical cassette. This is important because diffraction spot on a flat image plane are tangentially extended along the 2θdirection and for this reason, data at 2θ>60 deg are not accurate. With a cylindrical imaging surface, however, diffraction spots, especially in the equator direction, are not affected by the 2θ angle.

3. Outline of Galaxy

"Galaxy" is a totally new type of fully-automatic, Weissenberg data-collection system (Sakabe et al. 1997) installed at BL6C (Sakabe, 2000) at the Photon Factory. Galaxy consists of a rotated-inclined focusing monochromator (Watanabe et. al. 1999), a screenless Weissenberg-type camera, a pair of cylindrical (400mm radius) IP-cassettes in each of which a 450mm × 2513mm IP is fixed, a high speed IP-image-reader with five reading heads, an IP-image-eraser incorporating 8 160W sodium lamps and the same number of 30W fluorescent lamps, a cassettes transportation mechanism, a console and a secure, high-speed, dedicated computer network linking the beamline with data processing computers and servers. The rotated-inclined focusing monochromator is used because it is very efficient over a wavelength range. The exposure area on the IP can be varied using two upper and two lower screens. In this way the resolution along equatorial axis can be selected in the range of 0.71 Å to 5.7 Å assuming 1.0 Å incident X-rays.
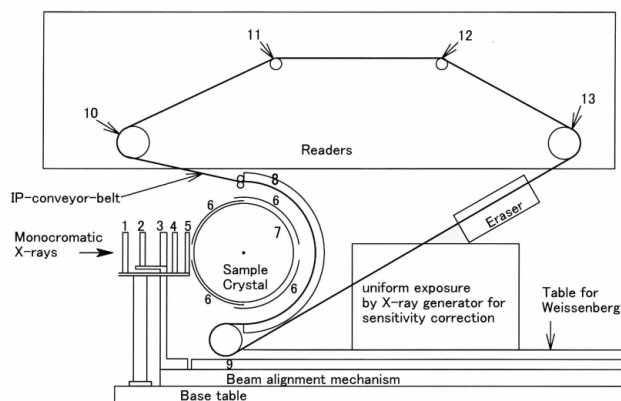
4. Design of Super Galaxy

Although Galaxy is a completely automatic data collection system designed for time-resolved macromolecular crystallography with monochromatic X-rays, both frame exchange rate and capability of a single IP cassette are insufficient data from which a highly refined structural mode can be derived. Toward the goal of overcoming these limitations we have designed Super Galaxy – a powerful instrument that should be installed on a very brilliant 3rd generation beamline in order that the full potential of the instrument be realized. A 3rd generation undulator beamline with a double crystal monochromator such as those in the PF-AR at KEK or at one of the other big storage rings would be appropriate.

The design of Super Galaxy is shown in Fig.2. Super Galaxy consists of a support structure, camera, 4 IP-image-readers, an IP-image-eraser employing UV-filtered sodium and fluorescent lamps, an X-ray generator designed to facilitate a flood field sensitivity correction, an IP-conveyor-belt system (Sakabe 1991) a controller and a high-speed, secure computer communications network linking the controller with the data processing computers and data servers.

4.1. The support structure

The support structure consists of a base table and a pair of quadrant slits (See Fig2, labels 1 and 4) for collimating the beam. A pair of position-sensitive ion chamber (PSIC in Fig.2) (Sato et al.

1999) are used to detect any spatial instability in the incoming photon beam and feedback to the quadrant slits 1 which move automatically in response. As the aperture of quadrant slit 2 is larger than that of 1, in practice, it is not necessary to move slit 2.

Figure 2

Schematic representation of Super Galaxy viewed from the side. 1,4; Quadrant slits, 2,3; PSPC, 5; Shutter, 6; Screens to select the frame size, 7; Removable helium chamber, 8; IP-cassette, 9; μ-rotation table, and 10-13; Head position of IP-image-readers.

4.2. Camera

The camera includes a stepping-motor-powered beam alignment mechanisms, the abovementioned PSICs which feedback to the alignment mechanism, a beam shutter (Fig.2, label 5), a set of screens to set the frame size (Fig.2, label 6), a half cylindrical IP-cassette (Fig.2, label 8) positioned on the table (Fig.2, label 9) for Weissenberg-mode operation and a removable helium enclosure (Fig.2, label 7) in which are located a collimator, a goniometer, a beam stop, a telescope, and a cryo-cooling nozzle. A flow cell and light source needed also to be installed in the helium enclosure.

4.2.1. The IP-cassette and set of screens to select a frame size

As the IP-cassette is a half cylinder, maximum acceptance angle is 180° corresponding to a crystallographic resolution of 0.71Å with 1.0Å X-rays. The maximum resolution in the horizontal direction is 1.58Å for 1.0Å X-rays. The maximum translation of the cassette along the oscillation axis (θ-axis) in Weissenberg mode is 100mm. As many protein crystals diffract to only resolutions lower than 2Å, screens are used to limit the frame size and determine the maximum resolution in the equatorial direction. This function is useful to reduce unnecessary use of disk space in the data server. Fig.3 shows an example of how the diffraction image area is selected by the two upper and two lower screens. There are two selection modes for the exposure area, namely, a symmetric setting (open angle:-2θ~+2θ) and an asymmetric setting (open angle:0~+2θ). Fig.3 shows an example of a symmetric setting. In the asymmetric setting, only the upper-side diffraction spots are recorded. One of the major advantages of Super Galaxy compared with Galaxy is that there are no windows in the X-ray beam path. Thus, the back scattering and the deficit image problems are removed from consideration. Also, the frame size can be adjusted freely in the equatorial direction.

4.3. The IP-image-readers and an IP-conveyor-belt

In contrast to the movable fully cylindrical IP-cassette and transportation mechanism of Galaxy, Super Galaxy employs a fixed half-cylindrical cassette and an IP-conveyer-belt The conveyor belt passes across the front side of curved cassette, through the four flat type IP-image-reader heads that are positioned in series and equally separated from each other by the distance corresponding to the maximum frame length, through the IP-image-eraser and X-ray exposure section used for correcting for sensitivity variations between the readers and for checking the linear response, and back to the cassette.
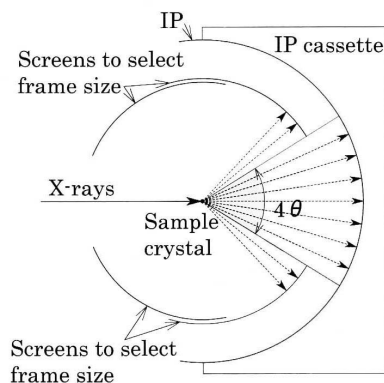
Figure 3

Symmetric setting of the screens to select the frame size.

Super Galaxy has a vacuum line and a pressurized gas line that are connected to the back of the cassette via electrically controlled valve to rapidly fix and/or release a section of the IP-conveyor-belt to or from the half cylindrical cassette. When the recording of a diffraction image is finished, the IP is quickly released from the cassette by switching from the vacuum line to the pressurized line. The IP-conveyor-belt is then moved on by a distance corresponding to length of the exposed frame as fast as possible. Such operations are successively repeated until either the data recording is completed or all the available space on the IP-belt is used. In the event that the recorded images exceed the length that can be accommodated by the 4 IP-reader heads (Fig.2. label 13), the eraser will be automatically switch off, and exposure continues until all available space on the IP-belt is used. The images are then read off by the 4 readers. The total number of frames recorded before reading is very important for time-resolved crystallography as mentioned at the session 2.2. Table 1 shows the relationship between the opening angle in the equatorial direction and the maximum numbers of frames that can be recorded before reading. It is an advantage of the IP-conveyor-belt method that the maximum numbers of frames that can be recorded before reading is proportional to the length of IP-conveyor-belt. This means that the number of frames can be freely varied to suit a any particular experiment. A linear encoder on one side of the IP-conveyor-belt determines the absolute position of each frame.

4.4. X-ray generator for corrections

Super Galaxy is equipped with multiple IP-image-readers and the inherent differences in sensitivity between these must be minimized and normalized for in order to get accurate data. It is also important to calibrate the linear response of each read head output versus X-ray dose. For this purpose, a uniform exposure device incorporating an X-ray generator is installed on Super Galaxy. In order to ensure a uniform flood field, the X-ray tube is positioned to be more than 1m from the imaging plate. The X-ray tube can be oscillated along the horizontal axis of the conveyor-belt. The distance that the X-ray tube

moves must be greater than the summation of the width of the IP and X-ray spot size in the direction of motion. The speed of the conveyor belt progression must be correlated with the oscillation rate of the X-ray tube in order to ensure uniform exposure across the whole IP. To construct a linear response curve across a dynamic range of 10^6, a large range of X-ray dose is necessary. A large range of dose can be achieved by adjusting the IP-conveyor-belt speed and the power of the X-ray generator. A similar type of correction is carried out on Galaxy.

5. Comparison of Galaxy and Super Galaxy

The major advantage of Super Galaxy is the fast frame-exchange speed and the image storage capacity before reading. For example, a time-resolution of 12 sec with 4 data sets (A-setting) or 2 data sets (S-setting) can be achieved for a monoclinic crystal assuming the following conditions; resolution limit of 1.93Å for 1.0Å X-rays, an exposure time for each frame of 1.0sec and a frame exchange rate of 60°/ sec. When a zone axis of 140Å is aligned to the spindle axis, horizontal movable distance for the Weissenberg mode is 3.0mm for 1.0Å incident X-rays. Taking the coupling constant of the Weissenberg mode as 5°/mm, then the oscillation angle for each frame is 15°, this corresponds to 12 frames in total giving a time-resolution of 12sec.

Table 1

The relations between the opening angle, setting, resolution and maximum numbers of recorded frames before reading. Horizontal resolution limits; 1.74Å for λ=1.0Å X-rays, 3.14Å for λ=1.8Å X-rays.

Open angle	Setting A# or S#	Resolution limit along equator diffraction		Number of frames
		λ=1.0Å	λ=1.8Å	
25 deg	A	2.31 Å	4.16 Å	62 frames
30	A	1.93	3.48	53
40	A	1.46	2.63	36
40	S	2.88	5.18	36
60	A	1.00	1.80	26
60	S	1.93	3.48	26
90	A	0.71	1.28	17
90	S	1.34	2.41	17
120	S	1.00	1.80	9
180	S	0.71	1.28	8

A# : Asymmetric setting, S# : Symmetric setting

For a crystal of higher symmetry, the oscillation angle to be covered is smaller and as a result the time required to record a complete data set is shorter for given set of experimental conditions. Table 2 shows a comparison between Galaxy and Super Galaxy

6. Conclusion

Super Galaxy is a fully automatic Weissenberg data collection system with a half cylindrical cassette using an IP-conveyor-belt. Galaxy has two fully cylindrical IP-cassettes. As many as 5~10 times more reflections can be recorded on a single frame by using the Weissenberg method compared to the oscillation method. Thus the number of frames per data set for an orthorhombic crystal using the Weissenberg method was estimated to be only 4 for opening angle being 40° using S setting. (Sakabe,N., et.al. (2001)). That is, with a frame exchange speed of 60°/sec, a data set can be recorded in 4sec with Super Galaxy and all images can be read out after maximum nine sets of time-resolved data have been collected as shown in Table 1. Another advantage of both Super Galaxy and Galaxy is that they use a sheet of IP which has a large dynamic range (10^6), very wide active area that facilitates the speed collection of time-dependent data sets to very high crystallographic resolution.

Super Galaxy has many advantages over Galaxy (see Table 2). The biggest improvement is that the storage capacity limitation of Galaxy has been overcome by increasing the available recording area and by the utilization of multiple reading heads. Also, the frame exchange speed is about seven times faster than Galaxy and there are no cassette exchanges. On the basis of the above comparison and given the characteristics of CCD detectors described in 2.2, Super Galaxy is the best system for time-resolved protein crystallography using monochromatic X-rays. A typical example, time-resolution rate of Super Galaxy is 4sec as abovementioned, on the other hand that of CCD systems are 2min described in 2.2.

The appearance of Super Galaxy will open a new frontiers in Enzymology especially in irreversible reactions, namely, electron Enzymology and bio-nano-chemistry. The design of Super Galaxy will be completed by October 2003 and construction will take two years after funding has been granted.

Table 2

The comparison between Galaxy and Super Galaxy.

Items	Galaxy	Super Galaxy
Type of the cassette	Movable fully cylindrical	Fixed a half cylindrical
Cassette radius Cassette width	400 mm 450 mm	420 mm 700 mm
Windows on the cassette for incident X-rays	Every 10 deg	Nothing (Quite open for 180 deg)
The effect of back scattering	Strong,	Nothing
Max. 4θ angle	180 deg	180 deg
Number of frames before reading (1.9Å resolution with 1.0Å X-rays)	12 frames for (A) 6 frames for (S) Fixed	53 frames for (A) 26 frames for (S)
Frame exchange speed	8.6 deg / sec	60 deg / sec
Weissenberg (Z)	10mm	100mm
IP transportation method	IP-cassette is transferred	IP-conveyor-belt
Speed of IP transportation	Cassette exchange 4 min	IP-conveyor-belt max. speed; 1,000mm / sec
N of reading heads	Parallel 5	Series 4 (or more)
Method of reading	IP-cassette rotation	Conveyor belt translation
Range (X-ray photons)	2 or 3 ~ 10^6	1 or 2 ~10^6
Reading time	9 min (5+4)	3min
Method of erasing	IP-cassette rotation	IP-conveyor-belt translation
Speed of erasing	A few sec	A few sec
X-ray generator (sealed tube, Cu target) power	30kV, 1mA (fixed)	30kV, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50 mA

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