Current status of Ibaraki biological crystal diffractometer iBIX -Several examples of the measurement -

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Abstract. Since 2004, Ibaraki prefecture has constructed the TOF neutron biological diffractometer (iBIX) at J-PARC for industrial use. Since the end of 2008, Ibaraki University has operated iBIX in order to support the experiment of users and to improve the instruments. The diffractometer is designed to measure samples with their cell edges up to around 150Å. In the beginning of December in 2008, the basic optics, the support system of detectors and the three detectors were completed for the diffraction experiments. We have tried to measure the TOF diffraction data of several proteins and organic compounds in order to estimate the efficiency and characteristics of the diffractometer. The TOF diffraction data of Ribonuclease A can be measured under the following conditions, beam power: 20kW, pulse repetition: 25Hz, range of wavelength: 0.5~4Å, exposure time: 18.7 hours. About one hundred Bragg reflections could be observed clearly on all detectors. The reflection of 1.58Å in minimum d-spacing which \( I_{\text{obs}}/\sigma(I_{\text{obs}}) > 7 \) was observed. This result implies that the efficiency of the iBIX will become 50~100 times higher than that of the present high performance diffractometer BIX-3 in JAEA after 1MW operation of J-PARC.

1. Introduction

Hydrogen atoms and water molecules around proteins play a very important role in the stability of their three-dimensional structure and many physiological functions of them. Recently, the demand on the positional information of hydrogen atoms in a protein molecule has been increasing after many projects in the world which have analyzed thousands of protein structures. Neutron diffraction can provide an experimental method of directly identifying hydrogen atoms in proteins as a technique complementary to ultra-high-resolution X-ray diffraction.

Recently, development of neutron imaging plate (NIP) and the diffractometer equipped with NIP on reactor neutron sources make a breakthrough event in neutron protein crystallography and meet the demand on structural biology in life science field [1]. Although the demand on neutron structural biology in the current life science field has continuously increased, neutron crystallography still remains to this day a severely limited technique.

To meet the increasing demand on neutron structural biology, Ibaraki Prefectural Government in Japan has started to construct a new high performance TOF neutron single crystal diffractometer
(IBARAKI Biological Crystal Diffractometer: iBIX) at the 1MW pulsed neutron source in J-PARC for industrial use since 2004. The diffractometer is designed to measure samples with their cell edges up to around 150Å with a resolution up to 1.2Å in case of biological macromolecules, and a resolution up to 0.7Å in case of organic compounds. The efficiency of the diffractometer would be achieved more than 100 times better than the present high performance diffractometer, BIX-3 (JRR-3, JA EA, JAPAN) [2]. Since the end of 2008, Ibaraki University has operated iBIX in order to support the experiment of users and to improve the instrument. In the beginning of December in 2008, the basic optics, the support system of detectors and the three detectors (a two dimensional position sensitive detectors of ZnS:Ag/10B2O3 scintillator with wavelength shift fiber system) [3]. In this paper, the current status of iBIX and several examples of measurement are presented.

2. Current status of iBIX
To realize the performance, iBIX has been installed on a coupled moderator which provides more intense peak and integrated intensity of neutrons flux but wider pulse shape than a decoupled one. The optic parameters (beam divergence and the distance between sample and detector: L2) have been determined to consider the overlapping of Bragg spots by using the original simulation program[4]. A super-mirror guide tube of iBIX was designed based on the determined optic parameter [2]. The curved guide tube can protect samples from high energy γ-ray and neutrons without any T0 chopper and the well designed tapered guide tube can serve to reduce the reflection number of neutrons on the surface of the mirror so that more neutrons arrive at the sample position. To realize high efficiency, iBIX requires a completely new detector system. A 2-dimensional position sensitive detector using ZnS:Ag/10B2O3 scintillator with wavelength shift fiber system which has a high spatial resolution, a less dead area, a high counting rate and high efficiency were developed [3].

![Figure 1. Diffractometer of iBIX.](image)

Since 2004, iBIX has been started to construct at BL03, MLF, J-PARC. In March 2008, the basic optics, the beam line shielding and the experimental shielding house were completed for the commissioning of the beam line. In May 2008, we started commissioning of iBIX under a trial operation. On the 31st of May, we could observe the first neutrons with current-TOF detectors. Spatial- and TOF-profiles of the direct beam at the sample position were measured by using a NIP and a 3He detector. These results showed that a feature of the direct beam is as expected from the simulation based on the design of the supper mirror guide tube. At the beginning of December in 2008, the support system of detectors, which is radial arrangement of detectors, and the three detectors (2θ for the centre of detective area: #1=95.6°, #2=139.0°, #3=156.6°, respectively, A total solid angle subtended by a sample: 1.953%) were installed in the experimental shielding house for the diffraction experiment (Fig.1). Since the end of December in 2008, iBIX has been opened to users.

3. Several examples of diffraction by iBIX
Since the end of December in 2008, we have started to measure the TOF diffraction pattern of several proteins and organic compounds in order to estimate the efficiency and characteristics of iBIX.

### 3.1. Results of several examples for proteins and organic compounds

The results of several examples of TOF diffraction pattern measured by iBIX were shown in Table 1. As the curved guide tube of iBIX can protect samples from high energy γ-ray and neutrons, we can select intermediate band of wavelength by adjusting the phase delay of the tail-cutter installed near the moderator. It is possible to select the range of wavelength from 0.5~8 Å with about 4 Å band width of wavelength. The optimum ranges of wavelength were selected for each sample. In case of organic compounds, minimum d-spacing less than 0.5 Å could be achieved in PW12. In case of proteins, minimum d-spacing 1.58 Å could be achieved in Ribonuclease A (RNase A).

#### Table 1. Results of several examples of TOF diffraction pattern measured by iBIX

| Sample               | Crystal Volume (mm$^3$) | Cell Volume (10$^3$ Å$^3$) | Power (kW) | Exp. Time (h) | Wave length (Å) | d$_{min}$ (Å) |
|----------------------|-------------------------|----------------------------|------------|---------------|-----------------|---------------|
| Na$_2$UMP*1          | 9                       | 12                         | 20         | 10.0          | 0.5~4           | 0.70          |
| PW12*2               | 40                      | 12                         | 20         | 20.8          | 0.5~4           | 0.47          |
| BIPO*3               | 0.052                   | 17                         | 20         | 13.5          | 3~7             | 3.56          |
| Trypsin+BPTI*4       | 2.25                    | 783                        | 20         | 16.0          | 4~8             | 3.80          |
| RNase A*5            | 12.7                    | 63                         | 20         | 18.7          | 0.5~4           | 1.58          |
| Thrombin+BIV*6       | 6                       | 395                        | 20         | 31.2          | 4~8             | 2.73          |

*1: Organic compound, *2: protein, *3: sample provided by Prof. Sugawara, *4: data given by Prof. Ozeki, *5: data given by Prof. Kawano, *6: data given by Dr. Yamada

3.2. The results of TOF-diffraction pattern of RNaseA – the highest resolution of protein samples –

One of the measured crystals is RNase A (RNase A, Crystal Volume=12.5mm$^3$ a=30.4Å, b=38.6Å, c=53.4Å, β=105.8°). Measurement conditions are as follow, beam power: 20kW, pulse repetition: 25Hz, range of wavelength: 0.5~4Å (1$^s$ frame) and exposure time: 18.7 hours. About one hundred Bragg reflections could be observed clearly on all detectors. The reflection of minimum d-spacing in the TOF diffraction pattern of RNase A is shown in Fig. 2. The d-spacing of the reflection is 1.58 Å and $I_{obs} / \sigma (I_{obs}) > 7$. Although there were some counting fluctuations in the data, a clear Bragg reflection were observed. Indexing of the TOF diffraction spots will be carried out by using new data reduction software “STARGazer” developed for iBIX [5]. It is expected that we can recognize a reflection with a smaller d-spacing than 1.58 Å on the diffraction pattern of RNase A after indexing.
Figure 2. X-TOF slice map, X-Y slice map and TOF-profile of RNase A measured by iBIX with the detector #2 which is arranged at \(2\theta=137^\circ\).

3.3. Estimation of measurement efficiency for iBIX under 1MW operation of J-PARC

The measurement efficiency of iBIX under 1MW operation of J-PARC could be estimated from the results of diffraction pattern of RNase A as mentioned above section. This sample could provide high quality diffraction pattern which has minimum \(d\)-spacing 1.58 Å in measurement time 18.7 hours per a setting of crystal orientation even under the operation of low accelerator power in J-PARC (20kW).

The overall completeness of Bragg reflections was calculated using the original simulation program [4] in case of the diffractometer with 64 detectors and 10 measurement settings. The result of overall completeness is achieved over 90% in minimum \(d\)-spacing 1.2 Å. We can calculate total number of crystal settings for each number of detectors based on the simulation result (Table 2). The exposure time for one crystal setting was also estimated simply from the gain of accelerator power (Table 2). Consequently, the progress for measurement efficiency of iBIX according to increasing the accelerator power and the total number of detectors was estimated and summarized in Table 2.

Under 1MW operation of the accelerator and 30 detectors available, we can measure the full data set of the RNaseA sample which has a standard volume of 1mm\(^3\) in about 4 days. The estimation of the measurement efficiency as mentioned above was assumed that Laue group of crystal is \(3\). If Laue symmetry of a measurement sample becomes higher, we can expect that the total measurement time becomes shorter. Generally, the resolution becomes worse according to the increase of the unit cell volume. The total measurement time of the sample of a large unit cell volume becomes longer than that of the sample of a small unit cell volume.

Currently, this estimation implies that the measurement efficiency of the iBIX will become about 50~100 times better than that of the present high performance diffractometer BIX-3 after 1MW operation of J-PARC. More accurate estimation must be curried out after the accelerator power increases.

| Date       | Power (kW) | No. of detectors | Exp. time (h/frame) | Total no. of setting | Total exp. Time (days/sample) |
|------------|------------|------------------|---------------------|----------------------|-------------------------------|
| Feb., 2008~ | 20         | 3                | 18.7                | 10*(64/3)=213        | 166                           |
| Apr., 2009~ | 20         | 14               | 18.7                | 10*(64/14)=46        | 35.8                          |
| 2012?~      | 1000       | 14               | 18.7/50=0.37        | 10*(64/14)=46        | 0.72                          |
| 2013?~      | 1000       | 30               | 18.7/50=0.37        | 10*(64/30)=21        | 0.33                          |

Crystal volume = 1mm\(^3\) (Standard volume for iBIX), Total exposure time= 4days

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