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Neutron structure analysis using the IBARAKI biological crystal diffractometer (iBIX) at J-PARC

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The IBARAKI Biological Crystal Diffractometer (iBIX), a new diffractometer for protein crystallography at the next-generation neutron source at J-PARC (Japan Proton Accelerator Research Complex), has been constructed and has been operational since December 2008. Preliminary structure analyses of organic crystals showed that iBIX has high performance even at 120 kW operation and the first full data set is being collected from a protein crystal.

1. Introduction

Hydrogen, protonation and hydration play important roles in life processes at the atomic level. Neutron diffraction is the most powerful tool for exploring these roles because it can sensitively locate the nuclear positions of H atoms, even in their ionized states, and identify the orientations of the water molecules that hydrate proteins (Niimura & Bau, 2008; Blakeley *et al.*, 2008). Since Schoenborn first demonstrated neutron protein crystallographic data collection from myoglobin (Schoenborn, 1969), several other protein structures have been studied using neutrons (Kossiakoff & Spencer, 1980; Phillips & Schoenborn, 1981; Wlodawer & Sjölin, 1982; Mason *et al.*, 1984). Although the importance of locating H atoms is high, the application of neutrons in protein crystallography has been limited by the relatively weak flux of the available neutron beams. The ability to collect neutron crystallographic data from crystals of biological macromolecules has been greatly enhanced by the successful development of a new neutron detector, the neutron imaging plate (NIP; Niimura *et al.*, 1994). Two high-performance neutron diffractometers, BIX-3 (Tanaka *et al.*, 2002) and BIX-4 (Kurihara *et al.*, 2004), both of which incorporate NIP technology, have been constructed at JRR-3 at the Japan Atomic Energy Agency (JAEA), Japan. BIX-3 and BIX-4 enable the recording of higher angle diffraction signals with high efficiency because of the high signal-to-noise ratio arising from the monochromatic methods used and the large solid angle subtended by the sample. Several important and interesting results have been obtained using BIX-3 and BIX-4 (Niimura *et al.*, 2006; Ogo *et al.*, 2007; Chatake *et al.*, 2007; Ishikawa *et al.*, 2008; Yamaguchi *et al.*, 2009; Adachi *et al.*, 2009; Tamada *et al.*, 2009; Yagi *et al.*, 2009; Iwai *et al.*, 2009), even though it typically takes one to two months to collect a data set from a protein sample with a relatively large crystal volume of more than 1 mm^3. LADI and LADI-III at the Institut Laue–Langevin (ILL), France, which are also equipped with NIP technology, have allowed shorter data-measurement times owing to the use of quasi-Laue data-collection methods (Niimura *et al.*, 1997) and smaller crystal volumes of as low as

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$\sim$0.1 mm^3 owing to the use of full deuteration (Blakeley *et al.*, 2008), at the cost of data resolution.

The BIX and LADI instruments were built at reactor neutron sources. At Los Alamos Neutron Science Center, USA, the first station to use time-of-flight spallation neutron technology, called the Protein Crystallography Station (PCS), has been built. The PCS started operations at the end of 2002 and has shown efficient performance even at less than 100 kW accelerator power compared with diffractometers installed at reactor sources, because it can scan large volumes of reciprocal space at once and resolve neutron wavelengths in time (Langan *et al.*, 2004). This was recently illustrated when data were collected from haemoglobin at the PCS to higher resolution and in a shorter period of time than had previously been possible using BIX-3 at JRR-3 (Kovalevsky *et al.*, 2010). The success of the PCS led to the design of a macromolecular neutron diffractometer (MaNDi) that is planned for construction at the Spallation Neutron Source (SNS), USA (Schultz *et al.*, 2005; Coates *et al.*, 2010). A new diffractometer called iBIX has been built at the Material and Life Science Experimental Facility (MLF) at the next-generation spallation neutron source at J-PARC, Japan. iBIX (IBARAKI Biological Crystal Diffractometer; Tanaka *et al.*, 2009) is expected to greatly extend the capabilities for neutron protein crystallography by using time-of-flight pulsed neutron technology at a powerful spallation neutron source. In this paper, it is reported that iBIX performs as designed and some preliminary experimental results using proteins and organic compounds are presented.

2. Specification of iBIX

iBIX is located on BL03 in the first experimental room of MLF at J-PARC (Fig. 1). The diffractometer room is shielded biologically and contains detectors and a goniometer at the end of a guide tube. Data collection is controlled from an air-conditioned cabin on a mezzanine floor, which also contains the data-acquisition electronics. The basic design requirements for iBIX were (i) that it should allow data collection from samples with a maximum unit-cell parameter of 135 Å, (ii) that it should allow data collection to 1.2 Å resolution for biological macromolecules and to 0.7 Å resolution for organic compounds and (iii) that it should be possible to collect a full data set in 3–4 d for a crystal of 2 mm^3 in volume and/or in about one month for a crystal of 0.1 mm^3 in volume of a biological macromolecule.

To realise this performance, a coupled moderator (intense neutrons but a broad pulse in time resolution) was selected. This is because biological neutron crystallography is an intensity-limited technique and the pulse repetition rate of 25 Hz of J-PARC provides the advantage of a high-resolution measurement in time, which compensates for the broad pulse shape of the coupled moderator. To retain the time resolution, iBIX has a flight-path length of 40 m (L_1; the distance between the neutron moderator and the sample). The beam divergence was designed to be less than 0.20° at the sample position by the combination of specially designed guide tubes composed of super-mirrors with different reflectivities, which have a total length of 25 m, in order to meet the requirements of crystal cell dimensions and the observable minimum d spacing.

Perhaps the most important component of iBIX is its detector system, which has to have a high spatial resolution (less than $\sim$1 mm) for each detector at a distance of 0.49 m (L_2) from the sample position, a smaller nonsensitive area that allows the detectors to cover the large solid angle subtended by the sample, a high counting rate, low γ-ray sensitivity and so on. In order to meet these conditions, iBIX adopted a new compact two-dimensional detector system with a short time-resolution composed of scintillator sheets of ZnS:Ag/$^{10}B_2O_3$ to convert Bragg reflections of thermal neutrons into light and wavelength-shifting fibre (WLSF) arrays in the X–Y axes to receive the scintillation light two-dimensionally and to transmit it to the light guide (Hosoya *et al.*, 2009). At present, 14 detector units, each with a sensitive area of 133 × 133 mm, have been installed to make measurements more efficient

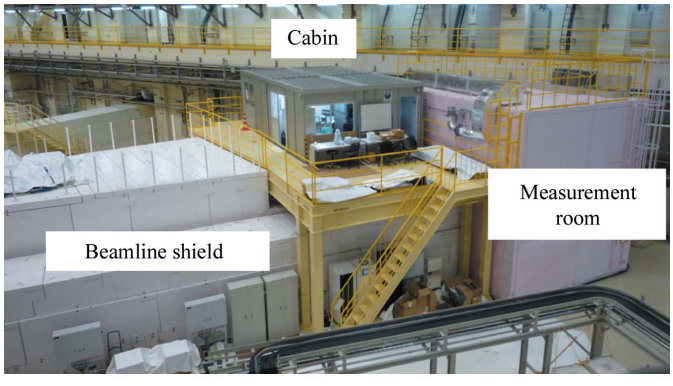

Figure 1
Appearance of iBIX: cabin (operation room), diffractometer room and beamline shield.

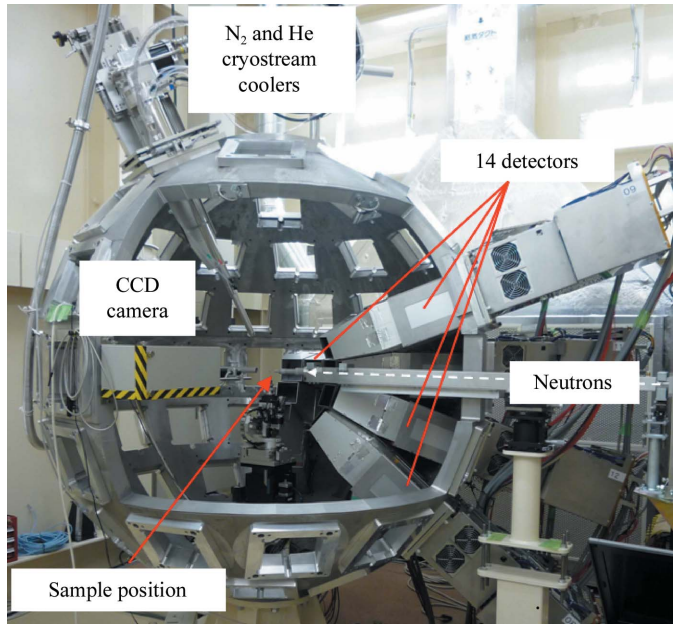

Figure 2
iBIX sample position and 14 detectors.

Table 1
Specification of iBIX.

Item	Specification
Planned accelerator power (MW)	1
Pulse repetition (Hz)	25
Moderator	Coupled H_2 (para) 100 × 100 mm^2
Choppers	1 tail cutter at L_1 = 8.5 m
Guide tube	25 m, 1-3Qc, tapered (Mirrotron, Hungary)
L_1 (m)	40
L_2 (m)	0.49
Measurement region in d spacing (Å)	0.35 < d < 50 (maximum unit-cell edge ∼150 Å)
Neutron wavelength range (Å) and flux	0.5 < λ < 8 or more (about 3.5 Å window), 7 × 10^7 neutrons s^{-1} cm^{-2}†
Detector	133 × 133 mm, 14 units, two-dimensional, scintillator, WLSF type
Detector spatial resolution (mm)	Less than 1
Standard size of sample (mm^3)	1, minimum 0.1 (1 MW)
Special sample environments	Cryostream of N_2 (90 K) and He (20 K)
Standard measurement time (d)	0.5 (organic compounds; 1 MW), 3 (biological macromolecules; 1 MW)‡

† Estimation between 0.5 < λ < 3.9 Å when operating at 1 MW. ‡ Sample volume of 2 mm^3 with 30 detectors.

(Fig. 2). The current parameters of the detectors are efficiency, 20–50%; spatial resolution, ∼1 mm; counting rate, about 500 kcounts s^{-1}; γ:neutron ratio, 10^{-5}. During every period of neutron beam time the incoherent scattering from a spherically shaped sample of V is measured by all detectors for less than 1 d in order to correct the detectors for variations in response. Rather uniform data is obtained by dividing the raw data by the correction data before data reduction.

The *STARGazer* data-reduction software, modified from *ISAW* developed at Argonne National Laboratory, USA, is used (Ohhara *et al.*, 2009). *STARGazer* is composed of a data-processing part and a data-visualization part. The data-processing part has fundamental data-processing functions and additional functions for real-space indexing and refinement of detector positions together with the orientation (UB) matrix and the identification of overlapping reflections from larger unit-cell samples. The data-visualization part allows the display of three-dimensional raw data in order to monitor data collection. It can also be used to confirm how well a preliminary UB matrix fits the raw data. In a recent experiment, it has been demonstrated that *STARGazer* can be used to index a lysozyme crystal in the tetragonal form with a maximum cell edge of 80 Å.

Cryogenic equipment is available for providing low-temperature gas [He (20 K) and N_2 (90 K); Fig. 2] for low-temperature data-collection experiments. N_2 can be supplied from air by compressing it, so the 90 K temperature is maintained without using N_2 cylinders, while the 20 K temperature is maintained by changing a He cylinder. The specifications of iBIX are summarized in Table 1.

3. Results

iBIX has been available to users since the end of December 2008 and several proteins and organic compounds were tested while the accelerator power was at around 20 kW. Diffraction data were obtained from sample crystals of organic compounds. One of them, ammonium hydrogen tartrate crystallized in heavy water, provided a full data set under cryoconditions (110 K). The crystal volume was 21.6 mm^3 and the unit-cell parameters were a = 7.65, b = 7.79, c = 11.04 Å in an orthorhombic form. The exposure time was 12 h per setting

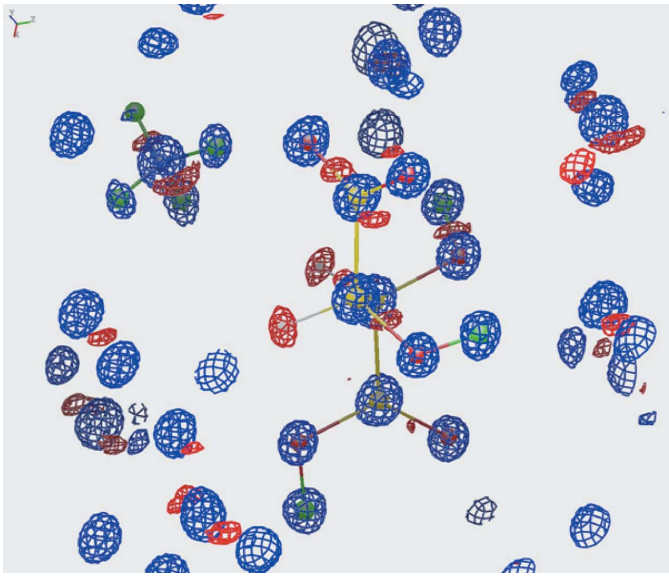

Figure 3
A $2F_o - F_c$ nuclear density map of ammonium hydrogen tartrate incubated in heavy water. Blue and red contours show 3σ and −3σ levels, respectively.

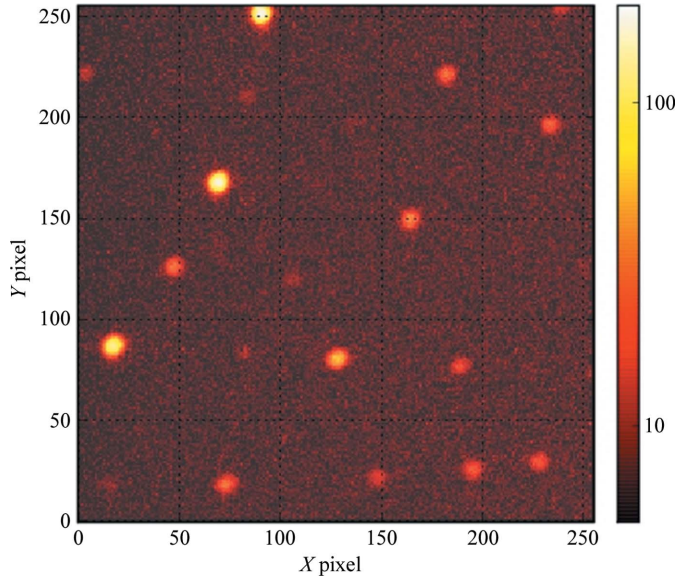

Figure 4
A diffraction image of RNaseA on one detector of iBIX. Intensity is corrected by the V data and shown on a logarithmic scale. One pixel corresponds to approximately 0.5 mm.

and the total measurement time was 11 d at about 20 kW accelerator power. A $2F_o - F_c$ nuclear density map of a preliminary structure analysis of ammonium hydrogen tartrate is shown in Fig. 3. Another organic compound, glutamic acid in the α phase, was also analyzed using a full data set collected on iBIX at room temperature. The crystal volume was 11.5 mm^3 and the unit-cell parameters were $a = 7.07$, $b = 8.76$, $c = 10.28$ Å in an orthorhombic form. The exposure time was 4 h per setting and the total measurement time was about 3 d at about 120 kW accelerator power. The final R factor was 8.89% for 1673 reflections with intensities greater than 4σ after application of an extinction correction in *GSAS* (*General Structure Analysis System*; Larson & Von Dreele, 2004).

Encouraged by these results, a full data set was collected from an RNaseA protein crystal treated in heavy water at room temperature using iBIX. This protein will act as a standard sample for neutron protein crystallography and has already been thoroughly analyzed (Yagi *et al.*, 2009). The crystal volume was 4.7 mm^3 and the unit-cell parameters were $a = 30.4$, $b = 38.6$, $c = 53.4$ Å, $\beta = 105.8^\circ$ in a monoclinic form. The exposure time was 5 h per setting and the scheduled total measurement time was about 17 d at 120 kW accelerator power. In Fig. 4, a diffraction image from 17 400 to 51 400 µs in time (from 1.7 to 5.0 Å in wavelength) obtained by one detector (located at 33° in 2θ) is shown. From these data, some diffraction spots at 1.4 Å resolution could be observed after indexing.

4. Conclusions and future prospects

A new diffractometer (iBIX) has been built at the next-generation neutron source at J-PARC and has started operation for users to perform measurements on crystals of proteins and organic compounds. According to the preliminary results presented here, the performance of iBIX at 120 kW accelerator power is very good. Because the planned final accelerator power at J-PARC is 1 MW and the number of detectors will be doubled within a couple of years, these results are very encouraging and suggest that we might expect nearly a 20 times higher efficiency than that at present when iBIX reaches its final conditions. We also expect further improvements in detector technology, as the detectors are still under further development, which will also increase the operating efficiency.

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