

OPEN ACCESS

10 years of protein crystallography at AR-NW12A beamline

To cite this article: L M G Chavas *et al* 2013 *J. Phys.: Conf. Ser.* **425** 012008

View the [article online](#) for updates and enhancements.

You may also like

- [Strain distribution in single, suspended germanium nanowires studied using nanofocused x-rays](#)
Mario Keplinger, Raphael Grifone, Johannes Greil et al.
- [Atomistic simulation for the size-dependent melting behaviour of vanadium nanowires](#)
Xiyuan Yang, Wangyu Hu, Fusheng Liu et al.
- [Determination of the stacking fault density in highly defective single GaAs nanowires by means of coherent diffraction imaging](#)
Arman Davtyan, Andreas Biermanns, Otmar Loffeld et al.



ECS
The
Electrochemical
Society
Advancing solid state &
electrochemical science & technology

DISCOVER
how sustainability
intersects with
electrochemistry & solid
state science research

10 years of protein crystallography at AR-NW12A beamline

LMG Chavas, Y Yamada, M Hiraki, N Igarashi, N Matsugaki, S Wakatsuki

Structural Biology Research Center, Photon Factory, High Energy Research
Organization, 1-1 Oho, Tsukuba, Ibaraki 305-0801, Japan

naohiro.matsugaki@kek.jp

Abstract. The exponential growth of protein crystallography can be observed in the continuously increasing demand for synchrotron beam time, both from academic and industrial users. Nowadays, the screening of a profusion of sample crystals for more and more projects is being implemented by taking advantage of fully automated procedures at every level of the experiments. The insertion device AR-NW12A beamline is one of the five macromolecular crystallography (MX) beamlines at the Photon Factory (PF). Currently the oldest MX beamline operational at the High Energy Accelerator Research Organization (KEK), the end-station was launched in 2001 as part of an upgrade of the PF Advanced Ring. Since its commissioning, AR-NW12A has been operating as a high-throughput beamline, slowly evolving to a multi-purpose end-station for MX experiments. The development of the beamline took place about a decade ago, in parallel with a drastic development of protein crystallography and more general synchrotron technology. To keep the beamline up-to-date and competitive with other MX stations in Japan and worldwide, new features have been constantly added, with the goal of user friendliness of the various beamline optics and other instruments. Here we describe the evolution of AR-NW12A for its tenth anniversary. We also discuss the plans for upgrades for AR-NW12A, the future objectives in terms of the beamline developments, and especially the strong desire to open the beamline to a larger user community.

1. Introduction

In the past few years, the field of protein crystallography has grown strongly as a necessary tool for numerous applications, as exemplified by the structure-based drug design. As a direct consequence, the number of researchers using MX beamlines has steadily increased, along with improvements in beamline equipment and technologies, and data processing and analysis.

Located on the campus of the High Energy Accelerator Research Organization (KEK), the Photon Factory (PF) storage ring has been operational for over 30 years, and the PF Advanced Ring (PF-AR) nearly as long. Although outdated by worldwide standards, both PF and PF-AR have benefited from a series of upgrades that have helped keep them strong and competitive. The insertion-device beamline AR-NW12A, the oldest macromolecular crystallography (MX) beamline presently operational at KEK, is located on the PF-AR North West section of the ring. AR-NW12A was constructed in 2001, and commissioned and opened to users in May 2003. Originally designed as a high-throughput MX station, AR-NW12A slowly evolved to accommodate a variety of experimental setups needed by an ever-increasing MX user community.

In the present communication, we give a short description of the evolution of the beamline, together with beamline use and performance statistics. Finally, an overview of the future implementations to be developed at the beamline will be discussed.

2. Beamline for high-throughput MX

2.1. Initial setup

AR-NW12A is one of the eight beamlines presently operational on the 6.5 GeV PF-AR, with a characteristic low emittance of $0.312 \times 0.029 \text{ mrad}^2$. The end-station was originally designed for high-throughput data collection with access to energy tuning for Multiple Anomalous Diffraction (MAD) experiments over a range of wavelengths – from 0.70 to 1.90 Å. By changing the gap of a vacuum undulator, the full spectrum of energy can be covered by shifting between the first and third harmonics, with nearly no beam intensity loss between the different energies (Fig. 1).

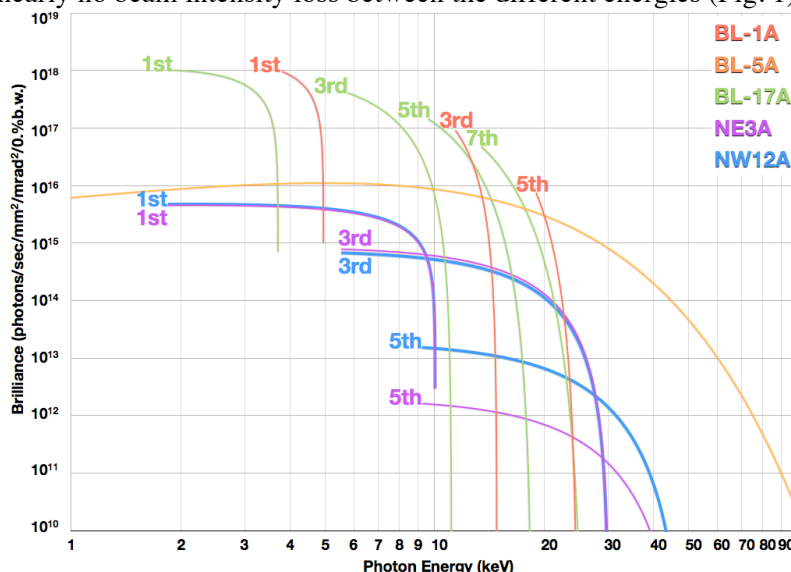


Fig. 1: Synchrotron radiation spectra available at the MX beamlines of the PF storage ring (2.5 GeV) and the PF-AR (6.5 GeV). Brilliance of the radiation vs. photon energy are denoted by red, orange, green, purple, and blue curves for the insertion devices SGU#01, MPW#05, and SGU#17 at the PF, and U#NE3A, and U#NW12 at the PF-AR, respectively. The spectral curves represent the peak positions for the first, third, fifth, and seventh harmonics within the allowable range of K parameters.

In a context of high-throughput data collection experiments, the X-ray optics were initially designed for the delivery of a 10^{11} photons/sec/mm² beam with a size of $200 \times 200 \mu\text{m}^2$ for the full range of available wavelengths [1]. In brief, AR-NW12A consists of a collimating mirror that brings the beam to the monochromator; a liquid-nitrogen cooled double-crystal monochromator, and a focusing toroidal mirror that horizontally focuses and delivers the beam to the sample positioned over 38 m from the source (Fig. 3). A good estimate of the efficiency of the beamline to provide a beam appropriate for structure determination using anomalous data can be seen by analyzing the number of coordinates deposited in the Protein Data Bank (PDB): over 29% of 683 coordinate sets of structures were solved using the MAD (or SAD) phasing method (Fig. 2).

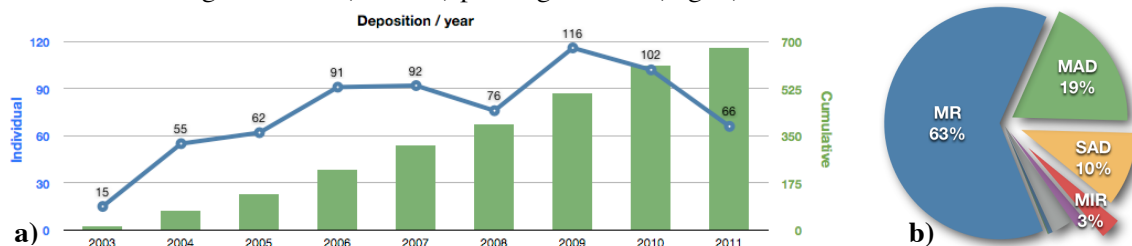


Fig. 2: PDB deposition at AR-NW12A. a) Number of coordinates deposited every year (blue curve) together with the accumulated PDBs deposited (green bars). The drop of deposition in 2011 can be

related to the Tohoku earthquakes. b) Distribution by structure phasing methods; the remaining 5% contain the direct method, SIR, and other undocumented techniques.

2.2. Optimization for high-throughput

While the amount of beam time available at AR-NW12A is fixed by the PF-AR operating time, the quantity of data recorded at the beamline has increased over the years as a result of small modifications to the beamline setup. The implementation of the PF Automated Mounting robot (PAM) and its Gemini double tongs [2] played a key role in the optimization of AR-NW12A for high-throughput. In the present setup, samples can be exchanged in a record time of 10 sec, allowing a greater number of samples to be mounted on the goniometer head in a shorter amount of time.

Importantly, the quality of data collected for solving a protein structure is particularly sensitive to the nature of the beam delivered. The beam needs to be homogeneous and consistent all along the data collection region. The single bunch nature of the PF-AR ring causes a decrease in current and beam intensity. As a consequence, the beam is slowly drifting over time, which clearly affects the quality of the recorded data, especially for weakly diffracting samples that require long exposure times and high redundancy. To overcome this issue, the implementation of a beam position feedback system [1] helped to stabilize the beam position by accounting for changes in the heat load on the monochromator, allowing the collection of homogeneous data without drastic intensity variations.

Finally, improvements in data handling and analysis, notably with automated data processing while the experiments are still running [3], minimize the loss of beam time during which no sample is exposed to X-rays, when users are analyzing their data before deciding whether to continue their experiments or not. Properly synchronized with automated data collection procedures and decision-making software, this optimization has the advantage of complete automation of sample handling, data collection and processing, reducing the time with no X-ray diffraction activity.

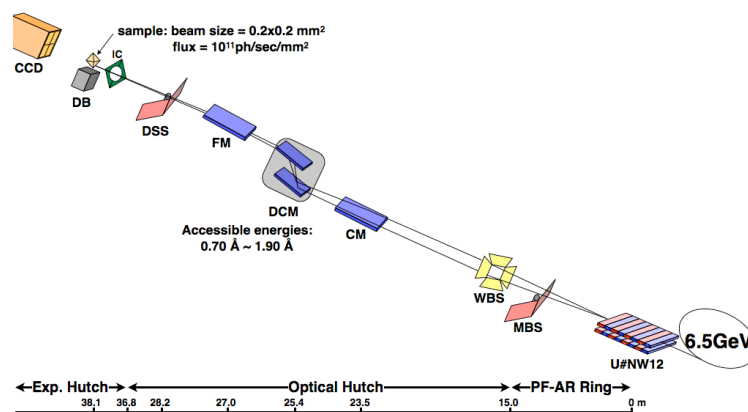


Fig. 3: Beamline layout with relative distances (not to scale) from the light source. U#NW12, in vacuum undulator NW12; MBS, main beam shutter; WBS, white beam slits; CM, collimating mirror; DCM, double crystal monochromator; FM, focusing mirror; DSS, downstream shutter; IC, ion chamber; DB, diffractometer box; CCD, X-ray CCD detector.

3. Beamline quality and efficiency

It is most difficult to judge of the quality of a beamline, mainly due to the fact that there are no concrete scoring procedures, and it is only possible to provide some statistics on how the beamline operates. A non-exhaustive list of the factors to take into account includes the number of publications per year, the average publication impact factor, the number of coordinate sets deposited in the PDB, the averaged beam time used vs. beam time lost, and the optimum beam size and intensity of the delivered beam at the available energies. Clearly, each beamline will have a different setup based on

the target science to be performed, which will affect each of the items of the list. Below we describe some of these features, in a view of the high-throughput character of AR-NW12A.

3.1. Publications

One straightforward way to judge whereas a beamline is used efficiently is to count the number of publications in which the beamline played a major role. This number of publications should be normalized by the total beam time made available to academic users. For the experiments performed at PF and PF-AR, the full list of publications for each beamline can be accessed via the PF-DataBase (PFDB)¹. As of July 1st, 2012, a total of 639 publications were reported for work performed at AR-NW12A. When normalized by the overall beam time opened to academic users (approximately 115 days per year since the beamline construction), an average of 1 publication is generated out of 1.8 days of X-ray experiments. Although not extraordinary, this number plays an important role in helping increase the level of the science performed at the beamline, and should be followed on a yearly basis.

Similarly, the impact factor distribution of the articles is a good indication of the quality and international impact of the experiments performed at the beamline. Figure 4 shows a chart of such a distribution for the full list of publications involving X-ray diffraction experiments performed at AR-NW12A, classified by the scientific journals' impact factors.

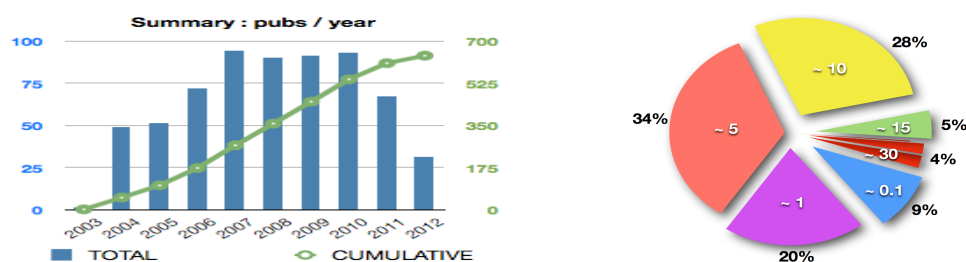


Fig. 4: A chart of the number of articles involving work at AR-NW12A registered to the PFDB since 2003 (left panel), and distribution of their impact factor (right panel). The drop in deposition in 2011 (left panel) can be related to the Tohoku earthquakes. The left panel shows a combination of the yearly deposition (blue) and the cumulative articles (green).

3.2. Protein Data Bank measures

In the case of MX beamlines, a concrete way of judging the productivity is to analyze the number of coordinate sets deposited in the PDB.² As indicated above, a total of 683 coordinates have been deposited to the database (Fig. 2), which can also be normalized against the available beam time. Thus, an average of 1.7 days of X-ray experiments at AR-NW12A produce 1 set of coordinates worth depositing to the PDB. In addition to the yearly number of publications, this PDB deposition number should be followed annually to get an idea of the quality and popularity of the beamline.

3.3. Beam statistics

The beamline can also be judged simply based on its physical and optical properties. However, whether the characteristics are a good fit will depend on the nature of the particular experiment. Thus, the beam delivered by AR-NW12A is suitable for samples of large size, but not for crystals of sizes lower than 100 μm . The energy is tunable from 6.5 to 17.7 keV, with an energy resolution of 2.5×10^{-4} . Through the feedback system mentioned above, the beam position at the sample is stabilized within 20 μm . Finally, a typical experiment on a macromolecular crystal of reasonable size (200 x 200 x 200 μm^3) exposed to the X-ray beam for approximately 2 sec per 1° oscillation exposure results in a full 180° data collection in less than 10 minutes.

¹ <http://pfwww.kek.jp/publications/pfdb.html>

² <http://www.rcsb.org>

4. Conclusions and future plans

AR-NW12A has been successfully running for 10 years as a high-throughput beamline for macromolecular crystallography. Since its opening, the beamline has been supported by two Japanese national projects for structural biology, including the *Protein 3000* project, and the lately completed *Targeted Proteins Research Program* project, during which it has evolved to allow a greater amount of data to be collected in a shorter amount of time, making AR-NW12A a highly automated beamline. In the latest plans of the *Platform for Drug Design, Discovery and Development* national project,³ the beamline will be upgraded for allowing multipurpose experiments.

Among the new experimental setups to be available at the end-station, high-pressure crystallography (HPC) using Diamond Anvil Cells (DAC) has been successfully reported [4]. To open HPC to the wider community, several difficulties in sample manipulation need to be overcome. A vertical goniometer will improve the cone of confusion. Its combination with an on-axis viewing system will reduce uncertainty in sample positioning. Finally, a new design of the DAC sample holder is under consideration, for accessing larger Bragg-angles.

A survey of the user community and modern structural biology shows that it is evolving in the studies of more complicated systems that often grow into micro-crystals. The manipulation of samples of smaller sizes at AR-NW12A would be a challenge, necessitating a better focusing of the X-rays, possibly by the addition of collimating slits. Although relatively low in intensity, even lower energies could be reached through the undulator's first harmonic. The addition of new phasing techniques, taking advantage of UV-light radiation damage is currently under intensive investigation. Finally, upgrading the X-ray detector could lead to even faster experimental times.

References

- [1] Chavas LMG, Matsugaki N, Yamada Y, Hiraki M, Igarashi N, Suzuki M and Wakatsuki S 2012 Beamline AR-NW12A: high-throughput beamline for macromolecular crystallography at the Photon Factory *J. Synchrotron Rad.* **19** 450-454
- [2] Hiraki M, Watanabe S, Honda N, Yamada Y, Matsugaki N, Igarashi N, Gaponov Y and Wakatsuki S 2008 High-throughput operation of sample-exchange robots with double tongs at the Photon Factory beamlines *J. Synchrotron Rad.* **15** 300-303
- [3] Yamada Y, Matsugaki N, Chavas LMG, Hiraki M, Igarashi N and Wakatsuki S Data management system at the Photon Factory macromolecular crystallography beamline *SRI 2012 proceedings*
- [4] Nagae T, Kawamura T, Chavas LMG, Niwa K, Hasegawa M, Kato C and Watanabe N 2012 High pressure-induced water penetration into 3-isopropylmalate dehydrogenase *Acta Crystallogr. D Biol. Crystallogr.* **68** 300-309

³ <http://www.pford.jp/>