R&D for Structural Genomics: A Fully Automated Crystallization and Observation Robot System "TERA" and "Sample Auto Changer" for SPring-8 Structural Genomics Beamlines

In the post-genomic era, there is great hope that protein research will reveal the structure and function of genome-coded proteins as live nano-robots. Structural genomics is one of the major post-genomic research fields engaged in determining the number of protein structures at the atomic level based on the information of full-genome sequences from various organisms, including humans from all over the world. At SPring-8, the Highthroughput Factory (Fig. 1) has been established as an integrated protein X-ray crystallography facility. Its investigations range from protein production to protein structural analysis using the synchrotron radiation (SR) source of the RIKEN Structural Genomics Beamlines, **BL26B1** and **BL26B2**. These beamlines are part of the Japanese structural genomics project, "TANPAKU 3000, National Project on Protein Structural and Analyses of Ministry of Education, Culture, Sports, Science, and Technology (MEXT)."

First, we are trying to overcome the bottlenecks in protein crystallization and data collection. However, in practice there remain many bottlenecks in high-throughput structure determination for structural genomics.

We designed and developed the full-automated crystallization and observation robot system, "TERA," by the oil-sitting drop method (Fig. 2) [1] based on pilot studies using a commercially available manual crystallization robot, IMPAX1-5 (Douglas Instruments, UK) [2] using the original initial crystallization conditions and a standard scoring system to attempt large-scale protein crystallization. We are currently conducting unique

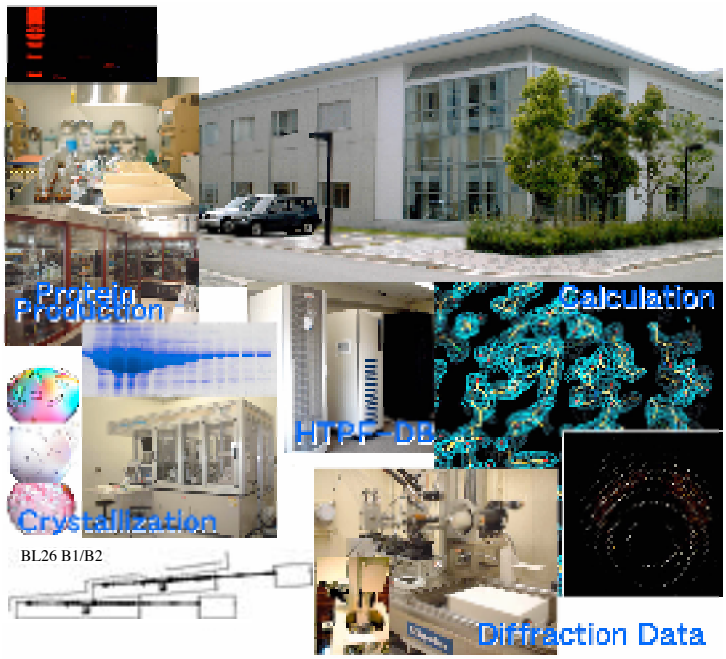

Fig. 1. RIKEN Highthroughput Factory (HTPF) for integrated protein structure determination. HTPF possesses full-functionality of SR crystallography, from protein production to crystallographic calculation. It includes RIKEN structural genomics beamlines with the integrated laboratory information system (HTPF-DB) developed in collaboration with HITACHI Software Engineering.

Fig. 2. Full-automatic protein crystallization and observation robot system, "TERA," jointly developed by Takeda Rika Kogyo, Stec and AdvanSoft. It has four components: (1) a liquid-handling robot for dispensing the crystallization setup robot at the right front, (2) microscopic photo system with CCD camera and XY plate loader at center front, (3) storage for 2,500 crystallization micro-plates and 125 crystallization reagent plates in the back, and (4) control software for managing operations with the data-base at left front. Close-up views: parts of the microscopic photo system (bottom-center panel) and the crystallization robot (bottom-right panel). Upper right panel shows various protein crystals.

crystallization experiments in the three screening steps: sparse initial, secondary for optimizing, and tertiary for additives [3].

The RIKEN Structural Genomics Beamlines have been designed to perform the high-throughput diffraction data collection for protein crystallography. In order to increase beam time efficiency and the ability to handle many crystals, the goal of this beamline development is the automated operation. During X-ray diffraction experiments, users would have to mount, dismount, centering and orientating many crystals. Therefore, a novel sample changing system has been developed to manipulate a number of crystals with the "Sample Auto Changer" as the core component (the left-hand in Figs. 3(a) and 3(b)) [4].

A small crystal of a few hundred microns or less is mounted on the originally designed plastic screw chips, called "Sample Pin," with a turnbuckle-like figure (Figs. 3(e) and 3(g)). The pin is screwed and mounted at the tapped hole of the goniostat head (the right-hand side in Figs. 3(a) and 3(f)). Contrary to a Hampton-like pin mounted on the gonio-head by a magnet, the orientation and positioning of the sample can be placed automatically at the X-ray beam center repeatedly after being aligned to the center by the operator. The mounted crystal is inserted into the tapped long tube the at arm-end of the Sample Auto Changer within 5 sec of changing the sample.

We can perform 24-hour X-ray data collection by using the Sample Auto Changer. This operation is controlled by the Beamline Scheduling Software (BBS) with a link *via* a unique crystal and tray ID to the Highthroughput Factory Database (HTPF-DB) system [5]. Accordingly, the information of the crystal sample, from the protein sequence to the structure analysis process, is obtained in the integrated and automated manner of TERA with supervision by HTPF-DB as well.

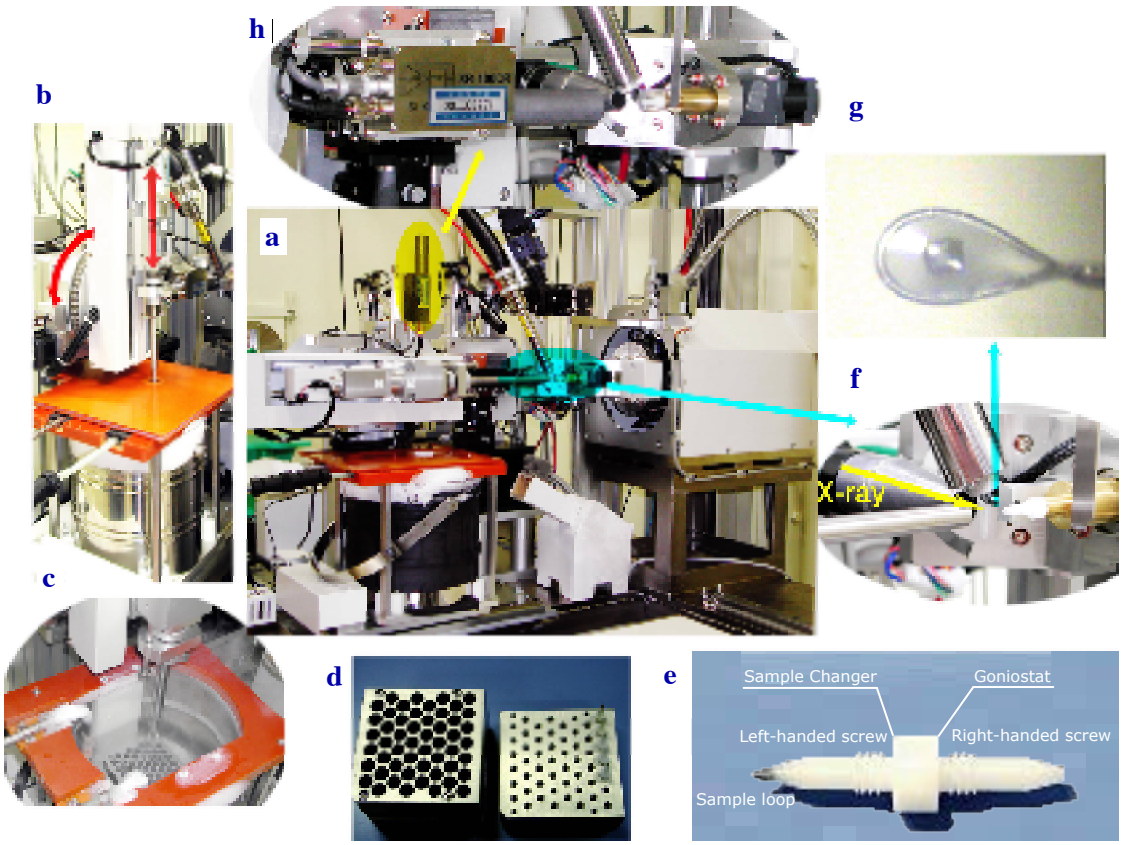

*Fig. 3. "Sample Auto Changer," a frozen crystal auto-changing system. **(a)** Sample Auto Changer and Auto Alignment Goniostat at BL26B2 station. **(b)** Crystal mounted on a Sample Pin (**e** and **g**) is picked from the tray (**d**) in liquid-nitrogen Dewer (**c**) and transferred to the goniostat head by Sample Auto Changer from left to right in panel (**a**), and its close-up view (**f**). **(e)** Sample Pin has left-handed screw and right-handed screw at each end with small nylon cryo-loop on the top for fishing the crystal. **(h)** Close-up view of XAFS measurement configuration.*

Koh Ida, Mitsuaki Sugahara, Masaki Yamamoto, and Masashi Miyano

SPring-8 / RIKEN

E-mail: yamamoto@postman.riken.go.jp and miyano@spring8.or.jp

References

[1] M. Sugahara and M. Miyano, Tanpakushitsu Kakusan Koso **47** (2002) 1026.
[2] N.E.Chayen *et al.*, J. Appl. Crystallogr. **23** (1990) 297.
[3] M. Sugahara and M. Miyano - in preparation.
[4] M. Yamamoto, G. Ueno, H. Kanda, K. Ida, T. Kumasaka, M. Miyano and T. Ishikawa - in preparation.
[5] H. Taka, R. Yoshida, E. Kanamori, Ishijima and M. Miyano - in preparation.