Acta Cryst. (2002). A**58** (Supplement), C302

DEVELOPMENT OF FULL AUTOMATIC PROTEIN CRYSTALLIZATION AND OBSERVATION SYSTEM AT SPring-8

M. Sugahara[1] K. Nishio[1] K. Kawamura[2] M. Nagashima[3] S. Yamai[4] K. Nonomura[5] S. Kuramitsu[1] S. Yokoyama[1] M. Miyano[1]
[1]RIKEN Harima Institute Structural Biophysics Laboratory 1-1-1 Koto, Mikazuki-Cho, Sayo-Gun HYOGO 679-5148 JAPAN [2]Takeda rika industory Co. Ltd. [3]Stec Co. Ltd. [4]Advansoft Inc. [5]System design Acty Co. Ltd.

Protein crystallization is one of the major bottlenecks in protein crystallography. Structural genomics project is expected to be produced a large number of proteins from various organisms every year, and so it will be needed to establish an efficient protocol to obtain suitable crystals for X-ray structural analysis and to develop a full automatic protein crystallization robot. A management system for a large number of data is also indispensable. A challenge to large-scale protein crystallization has started since April 2001 at Highthroughput Factory in RIKEN Harima Institute/SPring-8. In the first step, we made the standardization of protein crystallization procedure: (1) utilizing of a commercially available semi-automatic protein crystallization robot with micro-batch method, (2) establishment of efficient crystallization protocol composed of a set of crystallization screening conditions using each twenty-four precipitants and six buffer solutions, and (3) the evaluation standard of the crystallization-setting scoring. We have performed the preliminary crystallization trials with forty-three proteins from Thermus thermophilus HB8 using these conditions. Crystalline materials obtained from all proteins. Nine of them showed enough diffraction to solve their structures. Based on these results, we have designed and developed full-automatic crystallization and observation robotics, which is provided with liquid-handling and observation procedures for protein crystallization as well as plate stocking. The system can make it easy to manage score record and the date and time of crystallization setup.
The plasmids for the protein expression were provided by the RIKEN Structurome Project.

Keywords: HIGH THROUGHPUT CRYSTALLIZATION

Acta Cryst. (2002). A**58** (Supplement), C302

RIKEN STRUCTURAL GENOMICS BEAMLINES AT SPring-8 / OPERATION SYSTEM FOR HIGH THROUGHPUT DATA COLLECTION

G. Ueno[1,2] M. Yamamoto[1] T. Kumasaka[1] K. Ida[1,3] H. Kanda[1,4] S. Yasukawa[2] K. Sasaki[2] T. Ishikawa[1]
[1]RIKEN / SPring-8, 1-1-1 Kouto, Mikazuki SAYO HYOGO 679-5148 JAPAN [2]Rigaku co., Matsubara, Akishima, Tokyo, 196-8666, JAPAN [3]Yokohama City Univ., Suehiro, Tsurumi, Yokohama, Kanagawa, 230-0045, JAPAN [4]Shimane Univ. Nishikawatsu, Matsue, Shimane, 690-8504, JAPAN

RIKEN Structural Genomics Beamline I & II have been developed for high throughput protein crystallography (HTPX). The target of the beamline development is focused on automatic beamline operation, including sample auto-mounting, optics tuning, and automatic data collection. The required equipments for automatic beamline operation, such as sample mounting robot, remote-controllable optics, and a mechanism to exchange two types of area detector (CCD and IP) are controlled through a network of PC Workstations. The software for beamline operation has been designed based on Client/Server model that is suitable for job sharing and operation flexibility. The communication between client and server programs is established via TCP/IP socket connection. The main process scheduling whole experiments is running on the main workstation (BL Master), which communicates with the device servers running on other PCs assigned as controller of each hardware. The successive data collection of a number of crystals is processed using data acquisition format stored in a database system composed of a workstation, a server program, and storage devices.

Keywords: HIGH THROUGHPUT SPRING-8 STRUCTURAL GENOMICS

Acta Cryst. (2002). A**58** (Supplement), C302

STRUCTURE DETERMINATION OF A NOVEL PROTEIN OF UNKNOWN FUNCTION SYNTHESIZED USING A CELL-FREE SYSTEM

T. Wada[1,3] M. Shirouzu[1] T. Terada[1] T. Kigawa[1] S. Kuramitsu[2] S.-Y. Park[3] J. Tame[3] S. Yokoyama[1]
[1]Yokohama City University Protein Design Laboratory Riken Yokohama Institute Genomic Science Center 1-7-29 Suehiro-Cho, Tsurumi-Ku YOKOHAMA KANAGAWA 230-0045 JAPAN [2]RIKEN Harima Institute [3]Yokohama City University

A protein of unknown function from Thermus thermophilus HB8 (known as ID343) was synthesized using a cell-free system using *E. coli* transcription and translation machinery. The three methionine residues were replaced by selenomethionine (SeMet) and the crystal structure determined by the multi-wavelength anomalous diffraction method (MAD). This is the first case of a novel protein structure solved using the cell-free system. Native protein was also over-expressed in *E. coli*, purified and crystallized. The crystallization conditions of the SeMet and native proteins are different, the former giving crystals in space-group $P2_12_12_1$ and the latter space-group $P2_1$. The structure of the native protein was solved by molecular replacement using the model of the SeMet derivative. Refinement to 1.7 Å shows the two proteins are almost identical. The protein fold is highly similar to the CoA binding domain of succinyl CoA synthetase despite the sequence identity between these proteins being only 14%. Proteins produced by the cell-free system are therefore folded normally. This method of protein production is highly suited to high-throughput sample preparation for structural genomics.

Keywords: CELL-FREE SYSTEM UNKNOWN FUNCTION THERMOPHILIC

Acta Cryst. (2002). A**58** (Supplement), C302

RIKEN STRUCTURAL GENOMICS BEAMLINES AT SPring-8

M. Yamamoto[1] T. Kumasaka[1] G. Ueno[1,2] K. Ida[1,3] H. Kanda[1,4] M. Miyano[1] T. Ishikawa[1]
[1]RIKEN Harima Institute Coherent X-Ray Optics Laboratories 1-1-1 Kouto, Mikazuki SAYO HYOGO 679-5148 JAPAN [2]Rigaku co., Matsubara, Akishima, Tokyo, 196-8666, JAPAN [3]Yokohama City Univ., Tsurumi. Yokohama, Kanagawa, 230-0045, JAPAN [4]Shimane Univ., Nishikawatsu, Matsue, Shimane, 690-8504, JAPAN

The RIKEN high throughput beamline project for the structural genomics research started at the end of 2000. Two bending magnet beamlines are to be operated with simple and maximum efficiency, in answer to a huge amount of beam-time requests from the structural genomics research. SPring-8 BL26B1& B2 were assigned to RIKEN Structural Genomics beamline I & II. The final goal of the beamlines is automatic beamline operation. The two Structural Genomics beamlines have the identical beamline design from the source point to end station. The SPring-8 standard optics design for the bending magnet was adopted for simple operation. The beamline optics with a fix-exit Si double crystal monochromator followed by a two-dimensional focusing-mirror can facilitate MAD-experiment with high-flux monochromatic X-rays. The target of the research and development is focused on the automatic beamline operation to maximize beamline efficiency. We are developing the sample management system, which composed of the sample auto-changer and the database system, for high-throughput data collection. The sample management system and the beamline operating system make possible to automatic data collection without any operators. The beamlines will be ready for user operation from autumn 2002. The concept of automatic beamline operation and the present status and results of RIKEN Structural Genomics beamlines will be presented.

Keywords: HIGH THROUGHPUT STRUCTURAL GENOMICS SPRING-8