The Synchrotron Radiation Protein Crystallography Facility at NSRRC

The sequence of DNA defines the series of linked amino acids that forms a protein, and the folding of the protein controls its function. One key objective of decoding a genome is to understand the functions of proteins. Structural genomic utilizes protein crystallography to determine the three-dimensional structures of proteins and try to understand the protein function. Such large-scale projects pose special requirements for a modern protein crystallography facility, in term of accuracy, speed, and the capability for multiple-wavelength anomalous diffraction (MAD) methods.

Synchrotron Radiation Protein Crystallography Facility (SPXF) at NSRRC was funded by National Science Council (NSC) through the National Research Program for Genomic Medicine (NRPGM) to support high-throughput protein crystallography. SPXF, operated by the Protein Diffraction Group, is to provide a complete spectrum of protein-crystallography capabilities from sample preparation and characterization to data collection and computation for structure analysis. MAD data, diffraction data from microcrystals, and conventional diffraction data can be collected with extremely rapid throughput. The SPXF is a "national core facility" which receives proposals from scientists working in academic, industrial, and governmental sectors of the country. The submitted proposals are subject to peer-review by a Proposal Evaluation Committee (PEC). Sixty-five percent of the available beamtime from the SPXF beamlines is allocated to independent investigators, and the remaining 35% is used by SPXF staff for instrument development and beamline maintenance.

In order to provide a competitive MAD capability, the beamlines were constructed based on a high-field (3.2 T) superconducting, multi-pole (28 effective poles) wiggler (SW6) that can illuminate up to three beamlines for simultaneous operation (see also Highlight article by C.-I. Ma). The magnetic field increases the critical energy from 2.14 keV for a 1.25 T normal conducting magnet to 4.82 keV. The 28-pole device with a period of 60 mm can provide an intense X-ray beam up to 19 keV, with a flux in excess of 10^{15} photons/s/0.1%BW/mm^2 over the whole energy range of the SW6. The brilliance of this insertion device is almost equal to a third-generation high-energy synchrotron X-ray source.

Three beamlines are built as shown in Fig. 1 with the following features: (1) full remote control, (2) full system monitoring and diagnostics, and (3) stable and reproducible optics producing high-ntensity beams over a large wavelength range. The central beamline (BL13B) operates over the energy range from 6.5 keV to 19 keV (1.92 Å to 0.65 Å). Optimized for MAD experiments, it is also suitable

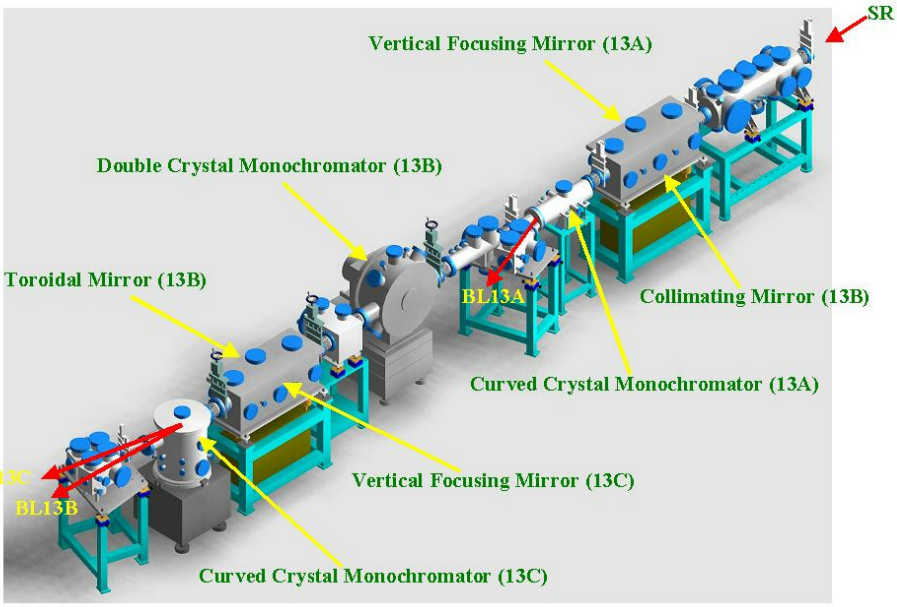

Fig. 1: Layout of the three high-throughput protein crystallography beamlines

for monochromatic crystallography. The principal components of the beamline include a front end, vertically collimating premirror, double-crystal silicon (111) monochromator with a fixed-height exit beam, and toroidal focusing mirror. The end station (BL13B1) is equipped with a state-of-the-art ADSC Quantum 315 CCD area detector for fast readout (< 2 sec) with motorized distance and both vertical and horizontal translation mounts, a high precision Huber single-phi axis goniometer, a Rigaku/MSC XStream 2000 sample cooling system (~100 K), a robotic sample changer for automatic sample mounting and centering, two dual-head SGI Octane2 workstations, high speed data network (100 MB/sec) with large data storage capacity (2 TB), user-friendly data acquisition software (BLU-ICE), and powerful data processing software (HKL 2000) used to collect single crystal diffraction data in high-throughput mode. Two additional fixed-energy beamlines (BL13C and BL13A) for monochromatic crystallography at 12.4 keV (1.0 Å), 12.7 (above Se-edge), and 13.5 keV (above Br-edge) are under commissioning. The components of these beamlines include a front end, vertically focusing mirror, and a horizontally focusing, single crystal silicon (111) monochromator. Like the central station, these side stations (BL13C1 and BL13A1) are equipped with similar instruments for high throughput crystallography.

The performance of BL13B MAD beamline is described in the following section. The energy range of this beamline from 6.5 to 19 keV can cover most absorption edges of the heavy atoms commonly used in protein crystallography (for example, Fe, Zn, Se, Br, Ho, Pt, Au, Hg, and U). The energy is tunable and has an excellent energy resolution around 2.2×10^{-4}, and can therefore benefit phasing studies of macromolecular crystals by using MAD methods. The focused spot size at 12.6 keV was measured to be 650 μm × 300 μm FWHM (h × v), similar to values calculated by ray tracing. The total flux measured at focus position without any aperture is shown in Fig. 2. At 12.4 keV, the measured total flux is almost equal to the calculated value. Protein crystallography experiments require a small beam, which is defined by different sizes of pinholes. The measured flux at focus position through a 200 μm aperture is also shown in Fig. 2. The useful flux at 12.4 keV is almost 100 times higher than that at BL17B, a 25-pole 1.8 T wiggler MAD beamline, and 10 times higher than that at BL12B, NSRRC bending magnet MAD beamline at SPring-8 in Japan.

A fast and large area detector is the key instrument for high-throughput crystallography, since it can substantially reduce the cycling dead time, and hence minimize the time-dependent effect of radiation damage on the sample, and therefore increase the quality of the data. To test the data quality produced by the ADSC Quantum 315 CCD area detector, the method reported by Z. Dauter, et al.

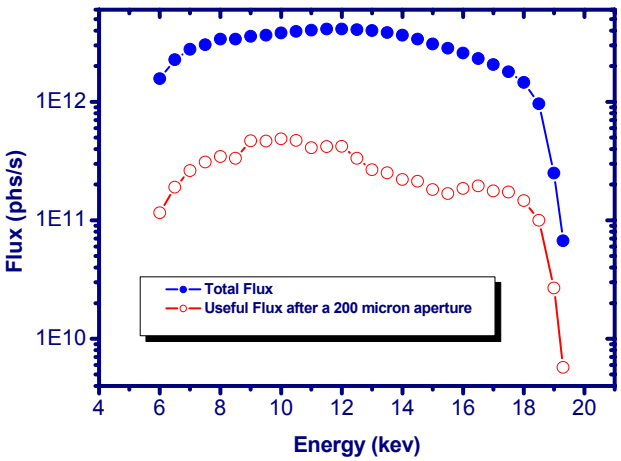

Fig. 2: Total flux measurement and useful flux measured after a 200 μm aperture of BL13B

(*J. Mol. Biol.* (1999) 289, 83-92) for solving structures of strongly scattering proteins using the anomalous scattering signal of sulfur was used. A major prerequisite for using this method is that the data are of extremely high quality because the "δf" term for sulfur is only 0.37 electrons and that for chlorine is only 0.47 electrons at 10 keV. A data set on hen egg-white lysozyme was collected using conventional data collection strategy without any special consideration to reduce the effect of radiation damage on Bijovet pair reflections. The data consist of 200 images with frame width 0.5°, which were processed with HKL2000. The maximum resolution is 1.4 angstrom, overall Rmerge is 5.0% with an average redundancy of 11.9, <I/s(I)> of 28.6, and $\chi^2 = 1.09$. The anomalous difference Fourier map shows significant peaks that correspond to the anomalous scatterers (S and Cl, see Table 1 and Fig. 3). The positions of each sulfur and chlorine are clearly marked by these peaks. Those features are only possible because of high-quality data. The result shows the excellent performance of the ADSC Quantum 315 area detector, which is well suited for synchrotron use.

Table. 1: Peak heights, I/s(I), for the lysozyme anomalous difference Fourier map

Atom	Peak Height	Atom	Peak Height
Met 105	14.22	Cys 127	8.34
Cys 30	12.55	Cl 204	11.24
Cys 115	12.07	Cl 201	8.09
Cys 94	11.73	Cl 202	7.75
Cys 64	10.77	Cl 205	7.69
Cys 76	10.76	Cl 203	7.60
Cys 80	10.35	Cl 206	7.41
Cys 6	10.15		

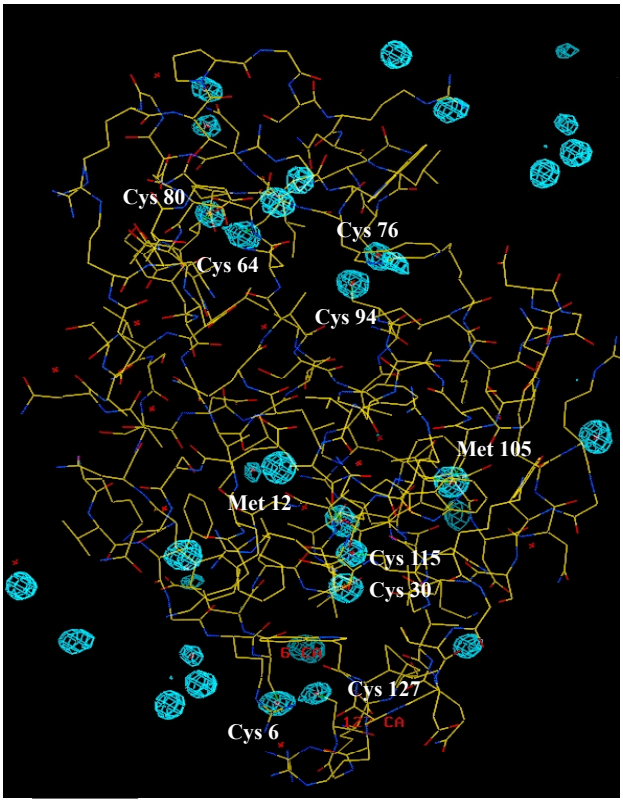

Fig. 3: The anomalous difference Fourier map of lysozyme using the coefficients ΔFanom and φcalc-90°

Because of the intensive data collection and data reduction in protein crystallography, a shared high-speed data network system will enhance the data acquisition throughput. This task is accomplished at the SPXF beamlines by a computing system consisting of six dual CPUs Octane2 workstations, four 4-CPUs Origin350 servers, one 8-CPUs Origin3800 computing server, two dual CPUs Win2003 servers, and a two terabytes storage disk array, running Clustered Storage Area Network (SAN) File System (CXFS) from SGI. The SAN offers the benefits of consolidated storage and a high-speed data network, while the CXFS enables true data sharing by allowing all SAN-attached system direct access to the same file system. The clustered file system provides data access speed well above what is achievable through traditional methods such as NFS and FTP, solving data sharing bottleneck for a broad range of environments.

With intense synchrotron sources and modern detectors, the data collection time becomes negligible compared to routine processes such as sample mounting, crystal centering, and data collection parameters determination. To make best use of the beam time, these routine processes should be fully automated. Users will also benefit from the automated data processing during an experiment. They are able to monitor the data quality and decide whether to collect further data or to stop the current experiment. Automation is an essential part of the high-throughput structural genomics programs. The Protein Diffraction Group will continue to develop high-throughput techniques, such as automated data collection as well as automated structure determination, and to upgrade the performance of the SPXF.

BEAMLINE
13B1 and 13C1 SW6 Protein Crystallography beamline

EXPERIMENTAL STATION
Protein Crystallography end station

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