X6A Scientific Advisory Committee Meeting
(February 23, 2009)

Report

General Comments

Over the past year continued emphasis has been the user community. X6A continues to be amazingly productive in terms of PDB deposits and publications by the various metrics presented at the meeting. They have carved out their user base at a time when there are an increased number of insertion device beamlines at the NSLS as well as at almost every other source in the United States. This appears to be due to User satisfaction and a simplified User access through a well established web based self-scheduling capability. Young faculty and new users are continuously attracted through emphasis on personal attention, teaching, and expert discussions.

User Program. Use and operation of X6A over the past calendar year was outstanding. In addition, reflecting past operation, it had the highest number of publications (57) of all bending magnet lines, many in very high profile journals. 70% of the publications resulted from projects developed only at the beam line. Part of the success of the line is the efforts made by the personnel, not only to provide excellent support for traditional structural biology groups but also to encourage and train groups that had not used X-ray diffraction before their interaction with X6A.

X6A continued to improve their time request interface. One of the reasons for the high degree of utilization of this beamline, besides the dedication of the personnel to the users, is probably the ease of requesting and scheduling beam time. There are two modes to request beam time: (1) through the X6A Web page (preferred), or (2) through the NSLS Pass System as a General User either through a long term project or Rapid Access. There has been no request through the later mode, as users seem to prefer the ease of self-scheduling beam time.

Users can access the beam line in person, remotely (remote users control the beamline through the net) or virtually (virtual users, beamline staff collects data). The majority of the users come in person with some virtual users. No remote usage was reported. There was some concern about future possible difficulties imposed from the laboratory in regard to remote access. It was suggested that the staff investigate the possibility of

providing access to one or two of the beamline computers from the outside as is done at the APS as well as at Universities.

One additional attractive feature, is the access to insertion device beamlines X29 and X25, if need arrives during a data collection visit to X6A. Established on an informal basis two years ago, only 3 groups requested access to this facility and were fully supported by the X6A personnel on these beamlines in the last calendar year. It is also envisioned in the future that X6A users would have facilitated access to other life science beam lines, such as the solution scattering beam line, X9. Although this additional features are very attractive to the program, the committee is concerned with the distraction posed by this added features on an already thinly stretched beam line personnel. The X6A proposal review and auto-scheduling are unique in that *any* independently peer reviewed project will be awarded beam time on X6A. If access to insertion devices is awarded, this SAC recommends that same metrics are applied and clear guidelines be established.

The utilization of the beam time has been superb. Of the NSLS available time 75% was utilized by users and 18% by beamline personnel. This 18% was divided 68% for maintenance and improvements, 32% for beamline personnel projects, and 8% went unused.

Users can collect data on site or remotely. At the end of the experiment they can take home scaled diffraction data and in many cases an electron density map. More and more users are starting to take advantage of a virtual access to the beam line. Championed by Dr. Jakoncic, users either mail their samples or leave samples behind when the scheduled beam time was not enough to exhaust all the samples. To further expand and support this feature additional staff is needed.

Data obtained at X6A was acknowledged in 102 deposited coordinates in 2007 and 61 in 2008. These resulted in 55 publications, 20 in journals with impact factors > 6. The use of beam time per structure was 2 days and per publication 3 days. These are outstanding statistics.

Technical Upgrades. It is obvious from the stellar beamline productivity that the majority of the beam time was devoted to user operations. Despite this restriction, there were several major hardware upgrades in the past year. Dr. Stojanoff presented four significant improvements that had a positive impact on the beamline.

Equipment upgrade and infrastructure development: The most important was the purchase and installation of the Q270 CCD detector from ADSC, Inc. The manufacturers claim a three times greater sensitivity as compared to the Q210r or the Q315 with a full resolution readout time of 1.1 seconds. Ten user groups have commissioned this detector with its increased sensitivity and larger surface area, 270 mm square. Another of the improvements was the installation of a near- on-axis or in-line sample visualizer. The visualizer was purchased from Crystallogic. It will be used in the automated sample alignment as well as equipment alignment procedures. The last of the major hardware upgrades was the replacement of a stepper motor for the omega motion to an air-bearing. These upgrades are all relevant and important to the user program, in fact it keeps them up to par with most beamlines on third generation sources.

All these upgrades were completed with a loss of only two weeks of user beam-time during the month of November 2008. This is a remarkable accomplishment that reflects the commitment of the personnel to maximum utilization of this beamline.

Three technical projects were presented: a beam position monitor (BPM) developed in collaboration with Dr. Peter Siddons group, a prototype He path, and capillary minibeam optics in collaboration with the PXRR. Championed by a summer student (C. Owen, SUNY Buffalo) BPM showed promising results but the spatial resolution was still too large for practical use in the beamline. The group is aware of these problems and the next generation will be developed during the summer of 2009. The He path, championed by Dr. Jakoncic, has also shown promising results with significant gain in intensity for energies bellow 10 keV. The group will seek to fully implement this feature in a future upgrade but will need additional funding for the construction. The minibeam optics commissioning studies presented by Dr. Allaire made use of Bilderback capillary focusing optics installed on the X25 undulator beamline. A focused beam size of 17 um FWHM in the vertical and 27 um FWHM in the horizontal was achieved. This beam was used in test case studies on lysozyme microcrystals and another crystal with unit cell dimensions of 300A. In order to resolve the spots of the 300A cell crystal, the detector was moved to 250 mm. The change in the detector distance from 120 mm in the lysozyme diffraction pattern revealed a possible limitation to the use of the capillary optics. The inhomogeneity of the incident focused beam could be seen as a hole in the individual spots. More studies are needed.

Computing Resources and Software upgrades: These included significant modifications of the control software. Championed by Ms. Qian these modifications included the change to Galil motor controllers for the diffractometer, the addition of the use of the detector in binned or unbinned mode, and the capability of remote start-up. Significant

increase in the data storage capacity as well as in the overall computing power of the beamline was implemented. All workstations, beamline control and data processing were upgraded, plus data storage increased from 3 Tb to 7.4 Tb, and a Gb network installed.

Future Improvements. Several improvements that will render the beam line increasingly user friendly and efficient are planned for the next period:

1. Sample environment,

    - Sample illumination for visualization of smaller samples and autocentering routines
    - Beam stop (SSRL like) for dose limited experiments
    - Improved He flight path for improved signal
    - Sensors for improved remote access smart magnets (for automounter operation)

2. KB mirrors for improved intensity,

3. Encoders for the diffractometer table which will improved the alignment accuracy and feed back

Overall Comments. The beamline has been extremely productive, both in the number of publications as well as the number of hours of user time that it provided as a service. This success is a reflection of the dedication of the beamline personnel to the user and to the operation of the beamline.

Scientific Reports

X6A has developed a group of loyal users that consistently utilize this beamline for their research. The scientific presenters reported the results of excellent projects with publications in highest impact journals. Some of these projects appear to have benefited significantly from the kind of attention that users receive in X6A. The interaction with the X6A personnel and their dedication was responsible for the success of many of the projects. Other projects have benefited from the ease of scheduling and use of the beamline for extensive studies of many crystal structures and complexes.

The following groups presented their results from X6A:

1) ***F. Jon Kull*** from Dartmouth College talked about a series of crystallographic studies performed at X6A using various phasing methods including multi-wavelength anomalous diffraction. First, he presented multiple structures of three kinds of kinesin motor proteins in complex with different nucleotide analogues. Conformational differences depending on the presence or absence of the γ-phosphate provide the link between the chemical ATP hydrolysis cycles and the mechanical cycle of motor movement. The crystal structure of an "atypical" kinesin family member known as NOD from drosophila showed that NOD may be catalytically active when not bound to the microtubule. This is different from most kinesins that require binding to microtubules for activation of their ATPase activities. He then showed 4 crystal structures of viral and host proteins involved in V. cholerae virulence, AphA, AphB, ToxT and HapR. Some of the viral virulence factors turned out to belong to the winged helix transcription factor superfamily. These structures provide basis for future novel drug development against cholera.

2) ***Emmanuel Skordalakes*** from the Wistar Institute, Univ. Penn, presented his exciting breakthrough on telomerase structural biology. Telomeres are repetitive DNA sequences at the 3'-ends of linear chromosomes and telomerase is responsible for his replication. It is a RNA-dependent DNA polymerase as it uses an RNA template for generating the repetitive telomere sequences. Telomere and telomerase are important for maintaining genome stability and is a drug target for killing cancer cells. His group recently succeeded in crystallizing the TERT core of the telomerase from T. Casteneum (Red Flour Beetles). The structure was solved by single isomorphous replacement with anomalous scattering (SIRAS) and refined at 2.7Å resolution. It revealed a structural architecture similar to the human immunodeficiency virus reverse transcriptase and other viral polymerases. A telomerase specific RNA-binding TRBD domain facilitates positioning of the template at the active Site of TERT. An interior cavity is observed in the structure of the TERT and can be modeled to bind RNA-DNA hybrid double stranded

nucleic acid. This result was published in Nature in 2008.

3) ***Jens Birktoft*** from New York University showed their novel DNA nanotechnology effort in designing three-dimensional self-assembled crystalline arrays. Using sticky-ended cohesion, they were able to create structures such as holiday junctions and three-dimensional DNA tensegrity triangle. The latter gave crystals in the size ranges of 100μm to 400μm in both native and iodinated forms (for phase determination) and diffracted to a reasonable resolution at X6A. The structure of an iodinated form was solved at 5Å resolution using single wavelength anomalous diffraction phasing. Additional results from three-dimensional triangles of different edge lengths were presented and shown that they may be used as rational macromolecular crystallization scaffolds.

4) ***Ming-Ming Zhou*** from Mount Sinai School of Medicine talked about their structural work on epigenetic gene regulation. In particular, he presented their recent work on the human protein Np95 and its SRA domain. He showed that the SRA domain of the Np95 binds hemimethylated CpG DNA, in addition to its interaction with methylated histone H3. The structures of both the SRA domain alone and its complex with DNA were solved using data collected at X6A and refined at 1.95Å and 2.45Å, respectively. The structure of SRA alone revealed a highly positively charged surface patch. Mapping of DNA binding of SRA by NMR showed that this surface patch undergoes chemical shift perturbation upon titration of DNA. Indeed, when the structure of SRA in complex with hemimethylated DNA was solved, it showed that the same surface patch is involved in contacting and recognizing hemimethylated CpG DNA. Remarkably, this recognition is achieved through a b

SUGGESTIONS FOR FUTURE DEVELOPMENT AND OPERATION

The X6A SAC members commend performance of the X6A beamline and hope for the continued commitment of the staff and the NSLS management in supporting an outstanding research program through personal attention, innovation and creativity.

The members of the SAC were pleased to learn that Wayne Hendrickson was chosen as the Director and Lisa Miller as the Vice-Director of Biological Sciences for NSLS II. The SAC found discouraging though that one option for the transference of X6A to NSLS II was not to locate it on an undulator beamline. The SAC felt that the NIGMS effort at the NSLS should be placed in one of the best insertion devices at NSLS II.