Workshop of the National Institutes of Health National Center for Research Resources and the National Institute of General Medical Sciences on Plans for Support of Future Life Science Synchrotron Research at NSLS-II

June 4 and 5, 2009

Bethesda, MD

1. Introduction and Background

A group of synchrotron structural biology and beam line experts (hereafter referred to simply as the ‘Panel’) participated in a workshop that was convened jointly by NIH NCRR and NIGMS to provide advice on the planning for structural biology beam lines and their operation at the National Synchrotron Light Source-II (NSLS-II). NSLS-II will be a high performance synchrotron light source which is currently being constructed by the DOE Office of Basic Energy Sciences (DOE-BES) at the Brookhaven National Laboratory (BNL) and is expected to become operational around 2015. A Glossary of frequently used abbreviations is given in the Appendix (Section 6.A). The Panel membership is given in Appendix Section 6.B. The Panel met for two days (June 4 and 5, 2009) in Bethesda, MD.

The Panel was asked by NIH to make recommendations regarding beam line optics and instrumentation specifications for future beam lines on NSLS-II, including the desired characteristics of the beam for each proposed scientific area, optimal insertion device characteristics and location and the potential utilization of wiggler-based sources. It was also asked to make recommendations for management models of NSLS-II Life Sciences beam lines.

The agenda for the meeting is included in the Appendix (Section 6.C). Representatives from NSLS-II presented overview and specific technical information relevant to the questions being considered by the Panel. This was followed by discussion and further questions for the NSLS-II representatives. Program staff from NCRR and NIGMS and the DOE Office of Biological and Environmental Research (DOE-BER) participated in the workshop as well.

This report is organized into six Sections. Following this brief Introduction and Background (Section 1) are three Sections that consider beam lines and instrumentation in the specific areas of macromolecular crystallography (Section 2), small angle scattering and diffraction (Section 3), and imaging (Section 4). Management considerations are discussed in Section 5 and appendix material is included in Section 6.

2. Macromolecular Crystallography (MX) Beam Lines and Sources

Summary of Recommendations:

- Brilliance (photons/s/mm^2/$mrad^2$/0.1%BW) is a key enabling driver for forefront MX experiments
- Build world leading beam lines for MX that fully exploit the unique capabilities of NSLS-II

- A low-β straight section provides the highest brilliance, but the possibility of a "focused" long straight section should be considered if it provides higher brilliance
- A pair of canted-undulator beam lines for MX is strongly preferred over investing in three pole wiggler beam lines
- The largest cant angle possible, 2 – 5 milliradians is very desirable
- The smallest horizontal beam size possible is very desirable
- A convergence/divergence of 500-1000 microradians at the sample position is important
- Design should incorporate a longer period undulator (21 or 22 mm) to avoid tuning curve gaps and minimize power load jumps when switching harmonics
- An x-ray energy range of 3.5 – 20 keV should be readily accessible, with the possibility of energies up to 30 keV

Macromolecular Crystallography and a Future Perspective. Macromolecular crystallography (MX) utilizing micron-sized samples (microcrystallography) has become a highly desirable tool for structural biologists. It enables structure determination from crystals with micron-dimensions by reducing the size of the beam to that of the crystal, thereby reducing the background and improving the signal-to-noise of the data. A micro-beam can be used as a probe to create a diffraction quality map in a larger crystal enabling selection of the best parts of the crystal for data collection. Micro-beams also allow for "walking the beam" along long crystal rods of small cross section, collecting partial data sets that can easily be merged into a full data set with reduced radiation damage. The high brilliance source properties of NSLS-II will provide unique opportunities for the construction of MX beam lines with micron or sub-micron x-ray beams as it allows for more of the emitted flux to be focused into a micron sized beam than existing synchrotrons. These beam lines should be world-leading, unique resources providing advanced capabilities for studying micro-crystals, large heterogeneous crystals, and potentially for reduced radiation damage during the data collection process.

Although the microcrystallography technique was pioneered at ID-13 of the ESRF approximately 10 years ago, beam time for MX was very limited as this station was not dedicated to biological studies. Since that time, microcrystallography capabilities have been developed at several synchrotron sources around the world driven by an increasing demand for such capabilities. Beam lines at the APS and ESRF now provide dedicated MX microbeam facilities offering beam sizes in the 5-10 micron range and flux in the 0.5 – 5 $x10^{11}$ photons/sec range by trading-off flux for beam size (utilizing apertureing in combination with focusing). Other facilities offer MX microbeams beams of varying sizes: CHESS (20-microns), Diamond Light Source (5-microns), Swiss Light Source (5x25 microns), and SSRL (7x70 microns) and apertures can again be used to match beam size to sample. The newly rebuilt PETRA-III storage ring at DESY in Hamburg will offer comparable performance and capabilities with NSLS-II as at least three high performance beam lines are planned for MX there (the emittance of PETRA-III of about 1.0-nm-rad is comparable with the initial design goals of NSLS-II).

Radiation Damage and Microcrystallography. For the low-Z atoms biologically relevant in MX (*i.e*., C, N, and O), the x-ray cross section tends to be dominated by the photoelectric effect. Most of the x-ray energy is converted into kinetic energy of the photoelectrons. As the photoelectrons traverse the crystal, they gradually lose more and more energy until they are recaptured. This re-deposition of energy is thought to be one of the major sources of radiation damage to the crystal. Several papers in the past few years have carried out both calculations and Monte Carlo simulations of photoelectron trajectory and energy loss. The emerging consensus has been that if the x-ray beam is small enough, then the photoelectron will carry the energy outside the beam footprint, resulting in reduced radiation damage where the beam impinged on the crystal. Recent studies at GM/CA-CAT at the APS with a 1–micron beam suggest that either sub-micron beam size and/or higher x-ray energy is needed to realize this reduction in damage in practice. The NSLS-II source properties will allow the beam to be focused to sub-micron dimensions with very high intensity; hence this reduced radiation damage effect may be obtained more routinely

MX Beam Lines and NSLS-II. The NIH "straw man" plan for MX beam lines at NSLS-II provided to the Panel as a basis for discussion suggested building three undulator beam lines for structural biology research on NSLS-II, with one dedicated to MX. The Panel felt very strongly that at the very least one undulator beam line should be designed and built and instrumented as a world leading beam line. A strong preference was expressed for building a pair of fully tunable, canted-undulator beam lines, thus making the best use of available funds and delivering very important added capacity for this important area of forefront MX research. These two recommendations are not mutually exclusive. The first of the canted-undulator beam lines must be world-leading and should not be compromised to fund the second beam line. The remaining funds should be used to build as much of the second beam line as possible, with additional funds sought to complete this beam line if required.

The "straw man" plan also mentioned the possibility of adding MX capacity by building multiple three-pole wiggler (TPW) beam lines. The bend-magnet radiation at NSLS-II has a relatively low critical energy; and therefore, the TPW concept was developed to increase the critical energy to ~6 keV. However, the strategy of building TPW beam lines for added capacity was not supported by the Panel. The intensity of an NSLS-II TPW is comparable to an APS bend-magnet beam line. Users have consistently shown a strong preference for the ID-based beam lines. A similar trend is seen at many of the other light sources having these capabilities. The Panel strongly felt that canted undulator beam lines were a much better investment than TPW beamlines.

The Panel was asked to comment on the choice of high-β or low-β sections for MX beam lines. The unanimous recommendation was to deliver the highest possible brilliance. The low-β section, as presented by NSLS-II staff, offers the highest brilliance option and is currently the only choice for canted undulators. However, Dr. Dierker suggested that a canted undulator design might be implemented on the high-β (long straight section), and with the addition of electron focusing optics in the center of the straight section an even smaller source size might be achieved. Based on the information presented in Dr. Shen's talk, the low- β section is the Panel's preferred choice. However, the option of a long straight with increased brilliance should be considered and NSLS-II staff could pursue design of this option as an alternative.

Canted undulator beam lines have been implemented at storage ring-based synchrotron sources to increase the capacity of beam lines per straight section. However, one must carefully consider the canting angle - ideally, one wants the largest cant possible to fully separate the two beam lines. Several factors such as cost, dimensions of the storage ring vacuum chambers, radiation shielding and available space on the experimental floor weigh in to limit the maximum angle. The APS implementation of the canted undulators incorporates a 1.0-milliradian cant angle between the two beams. The GM/CA-CAT dual canted-undulator beam line design uses sequential horizontal deflecting mirrors to further separate the two fully tunable beams. The implemented APS design was a balance between the highest energy available on the horizontally deflected beam line beam and the separation between the two beam lines. Although the separation is workable, implementation of a large detector such as the Pilatus 6M on the horizontally deflected beam line poses a challenge. The GM/CA-CAT canted undulator design is being replicated at PETRA-III which has a 5.0-milliradian cant angle, providing significantly more separation of the beam lines. The Panel recommends a cant angle at NSLS-II in the range of 2 – 5 milliradians to accommodate equipment such as larger detectors.

The small source size and low divergence of the beam at NSLS-II will allow the full central cone of undulator radiation to be collected and focused. For example, a pair of state-of-the-art mirrors that will be available in a few years (0.25 microradians RMS slope error) could be oriented in a Kirkpatrick-Baez geometry providing a focused beam of 5 x 2 microns (H x V, FWHM) with divergences of 720 x 120 microradians (H x V, FWHM).

The flux in the central cone of the APS Undulator A and NSLS-II IVU-20 should both be about 2×10^{13} photons/sec at 12 keV. To achieve a 5-micron beam, GM/CA implemented a “mini-beam collimator” that defines the beam size and reduces the intensity to 3×10^{10} photons/sec. At this flux density, the Henderson limit[1] will be reached in about 50 sec for a lysozyme crystal. By comparison, if the full flux of NSLS-II is focused to 5-microns, the Henderson limit will be reached in about 80 milliseconds! In principle, one could focus to 1-micron in one step at NSLS-II, but the divergence would exceed 3.5 milliradians. With the 1-micron beam at GM/CA, it takes about 40 seconds to reach the Henderson limit. If the full beam at NSLS-II were focused to 1-micron, then the time to the Henderson limit would be reduced to an extremely short 5 milliseconds, suggesting that one can reduce the divergence at the expense of flux by using an upstream slit to define a smaller source point or by multistep demagnification.

The spectral properties of the undulator source should be designed to cover a continuous x-ray energy range from 3.5 to 20 keV (with a possible extension to 30 keV). There should not be any gaps in this range and harmonic transition should avoid the absorption edge energies of the most common elements of biological interest. A list of the most common elements and their edge energies is shown below in Table 1. The

[1] the Henderson limit is 2×10^7 Gy (J/kg) and is defined as the X-ray dose a cryo-cooled crystal can absorb before the diffraction pattern decays to half of its original intensity.

tuning curve of the 20 mm period undulator has a gap above 4 keV which would prevent one from using L_3-edges of iodine and xenon for MAD phasing. The transition between the 1st and 3rd harmonic is the only one that causes a large heat load change on the beamline optics - all the other transitions can be accomplished with small power load changes. It also should be noted that transitions can be avoided for specific elements by remaining on the lower order harmonic with only a slight loss of intensity. The optimal undulator for MX might be a device with a 22 mm period as this would close the gap at 4 keV and further reduce the intensity (and power jump) at all transitions.

Table 1.*

Element	Absorption Edge	Energy (keV)
Mn	K	6.54
Fe	K	7.11
Co	K	7.71
Ni	K	8.33
Cu	K	8.98
Zn	K	9.66
Se	K	12.66
Br	K	13.47
Kr	K	14.33
Rb	K	15.20
I	L_3	4.56
Xe	L_3	4.78
Cs	L_3	5.01
Ta	L_3	9.88
W	L_3	10.21
Os	L_3	10.87
Ir	L_3	11.21
Pt	L_3	11.56
Au	L_3	11.92
Hg	L_3	12.28
Pb	L_3	13.04
U	M_5	3.55

*Note that the highlighted elements are the most commonly used for MAD phasing. Elements such as P and S have absorption edge energies that are not routinely accessible for MX.

3. Small Angle X-ray scattering and Diffraction (SAXS/D)

The ultra high brilliance beam characteristics at NSLS-II have the unmatched potential for supporting a world-leading beam line for non-crystalline x-ray scattering – including solution scattering, fiber diffraction and grazing incidence small angle x-ray scattering

(GISAXS). Capabilities of such a beam line would enable the most challenging classes of SAXS/D experiments, including those of a time-resolved nature, that are currently beyond the state-of-the-art.

Demand. An increasing number of structural biologists worldwide are turning to SAXS/D as an effective tool to complement high-resolution structural studies. Especially noteworthy is the ability of SAXS/D in particular to study conformational changes as a function of time under biologically relevant conditions. A state-of-the-art SAXS/D beam line on the NSLS-II facility will enable time-resolved studies on the order of microseconds to milliseconds, a time domain physiologically relevant in many macromolecular systems yet largely out of reach with today's capabilities. It is anticipated that the combination of substantial advances in microfludic control of samples, automatic sample feeds, increased impact of computational methods and scientific needs for the highest possible time resolution in the study of macromolecules and macromolecular complexes will drive greatly increased demands for state-of-the-art SAXS/D facilities. Experimental measurements such as those available from SAXS are also of particular importance for providing "benchmarks" for molecular dynamics simulations which have just recently achieved total simulation time beyond 10 microseconds. A properly configured ID beam line at NSLS-II will make possible entirely new classes of SAXS/D experiments, improving on existing limits for time resolution and improving on existing capabilities for the study of structure and dynamics using very small sample volumes.

Brilliance. The SAXS/D beam line should provide the highest possible brilliance along with the smallest possible beam size. This will enable the highest possible time resolution together with the smallest possible scattering volume. Anticipated advances in microfluidics and mixing chambers will make very high time resolution possible and use small sample volumes. The most efficient use of samples and highest possible time resolution using rapid mixing techniques will be aided by using the smallest possible beam cross section. Microbeam capabilities would also open up *in-situ* studies and potentially improve on the data quality in fiber diffraction and lipid GISAXS studies. Therefore, based on the information presented by the NSLS-II team, it is recommended that the scattering beam line be located on a low- β straight section of the ring. Very little compromise in beam divergence is expected since the NSLS-II low-β undulator provides a extremely low beam divergence which is comparable to, or lower than, currently world-leading small angle x-ray scattering facilities which have been primarily built on high- β sections to minimize beam divergence at the expense of larger beam size. It is the opinion of the Panel that smaller beam size takes higher priority for biomedical applications when the beam divergence is already extremely low.

Tunability. Energies for the SAXS/D facility should be tunable to allow use of anomalous SAXS (ASAXS). Although current use of anomalous scattering in SAXS measurements has been relatively limited, there are a large number of potential applications, and tunability across the same energy range as for macromolecular crystallography (*ca*. 3.5-20 keV) would provide comprehensive support for this capability. For example, recent developments in nano-particle/crystal labeling have become more practical and found increasing use in providing a "molecular ruler" between labels.

Anomalous scattering would be advantageous in minimizing cross-term between the macromolecule and label, hence extending the utility of this approach.

Compatibility with Canted Undulator and Hutch Location. The needs of a SAXS/D beam line are compatible with a canted insertion device only if experiments are performed in the downstream end station. Ancillary equipment such as lasers, FPLC, high throughput sample feeds and complex sample handling devices (for fibers and surfaces) have the potential for being substantially affected by the presence of a beam pipe in the hutch. It is hence very important and advantageous to have an unencumbered and fully dedicated experimental station which is possible only in the downstream location of a canted undulator beam line supporting two hutches.

4. Imaging

Biological imaging at NSLS-II will have significantly enhanced capabilities as a result of the extremely bright x-ray beams that can be delivered from the storage ring. A comprehensive biological imaging program will require beam lines that cover the range from the infrared to the hard x-rays. The extremely high brightness of NSLS-II will provide unique opportunities for x-ray imaging experiments on the micron and sub-micron scale.

The Panel recommends that high priority requirements for new, world-class facilities for imaging include:

- A coherent soft x-ray beam line covering the energy range between 0.25 – 2.5 keV including the water window as well as important absorption edges between carbon and sulfur. A full length undulator in a low-β straight section will be most appropriate as a source for this beam line. The beam line will support a program including coherent diffraction microscopy, STXM-based spectromicroscopy, as well as ptychography.

- A hard x-ray nanoprobe beam line for fluorescence mapping, XANES, and 3D fluorescence and absorption tomography. A zone plate instrument on a low-β straight section with an energy range of 2-15 keV (spectroscopy from P to Zn) and 30 nm spatial resolution would be well matched to the needs of the biological community. Construction of this beam line as the canted branch of the NSLS-II Submicron Resolution X-ray probe (SRX) project beam line would be a unique opportunity to fully leverage NIH funds since considerable infrastructure will already be built as part of the SRX project (*e.g.* hutches, transport lines, shutters, utilities, windows, white beam components and stands, gate valves, beam conditioning optics, personnel safety systems) and will strongly complement the SRX scientific program.

In addition, the Panel strongly urges that some of the best NSLS beam lines serving the biological imaging community be considered for transfer to appropriate locations at NSLS-II.

5. Management Considerations

The Panel commends the NSLS-II leadership for integration of life sciences research into the design of the new facility and for recruitment of visionary leaders to this effort. There is confidence that the life sciences leadership team (Wayne Hendrickson and Lisa Miller) will foster the development of facilities that are both suited to the biomedical mission of NIH and at the cutting edge of synchrotron science and structural biology. The Panel concurs with the plan for overall coordination of life sciences beam lines in which standardization is balanced with the flexibility required for innovation. There was a strong consensus that any beam lines supported by the NIH should be at the state-of-the-art, should expand the capabilities of existing synchrotron facilities, and should take advantage of the special features, particularly the very high brightness, of NSLS-II.

The model for coordinated management of life sciences beam lines has several advantages. Primary among these is the unified management of funding from multiple sources, while simultaneously respecting the goals of each funding organization. Substantial economies of scale and efficiencies of operation should be realized when staff can be deployed without regard to their funding source. This model has, for example, worked exceedingly well at SSRL and NSLS (where NIH and DOE-BER funds together support structural biology programs). It offers strong benefits to the user community through standardization of instrument control and user interfaces, simplifying training and facilitating cross-beam line utilization. An additional benefit to the user community is centralized management of proposal review and scheduling. Again, this approach simplifies processes from the user perspective, makes most efficient use of staff and optimizes facility utilization. Indeed, such centralized management of beam time proposals and allocation is essential given the DOE-BES requirement for 80% of beam time to be allocated to proposal-based experiments.

The Panel feels that the plan to develop a "biology village" at the NSLS-II also has major advantages in fostering a lively intellectual environment and in facilitating scientific and technical collaborations.

Centralized support of the development and operation of beam lines for the core techniques of macromolecular crystallography, small-angle scattering and imaging may be advantageous. An overall leader of life sciences facilities and a lead scientist in each of the three fields was the favored model in Panel discussions, although this is by no means the only route to success.

A process by which groups outside NSLS-II may develop and operate beam lines should be a good route to innovation. This mechanism may be especially beneficial in specialized fields, such as time-resolved crystallography or footprinting, in which the NSLS-II organization has limited expertise. Ways in which outside groups' proposals could be evaluated, in a standard peer-review setting, should be explored, which should factor into consideration the unique attributes that may be associated with such groups.

6. Appendix Information

A. Glossary

APS	Advanced Photon Source
BNL	Brookhaven National Laboratory
CHESS	Cornell High Energy Synchrotron Source
DESY	Deutsches Elektronen Synchrotron
DOE-BES	Department of Energy, Office of Basic Energy Sciences
DOE-BER	Department of Energy, Office of Biological and Environmental Research
ESRF	European Synchrotron Radiation Facility
FPLC	fast protein liquid chromatography
GM/CA-CAT	NIGMS/NCI-funded beamline sector at APS
GISAXS	grazing incidence small angle x-ray scattering
IVU	in-vacuum undulator
MAD	multiwavelength anomalous dispersion phasing
MX	macromolecular crystallography
NIH	National Institutes of Health
NCI	National cancer Institute
NCRR	National Center for Research Resources
NIAID	National Institute of Allergy and Infectious Diseases
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIGMS	National Institute of General Medical Sciences
NSLS-II	National Synchrotron Light Source-II
SSRL	Stanford Synchrotron Radiation Lightsource
PETRA-III	new (2009) high brilliance synchrotron radiation source at DESY
SAXS/D	small angle x-ray scattering and diffraction
STXM	scanning transmission x-ray microscope
SRX	submicron resolution x-ray probe
TPW	three-pole wiggler
XANES	x-ray absorption near edge spectroscopy

B. Workshop Participants - NSLS-II Meeting June 4 & 5, 2009

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Participants from Federal Agencies

Several interested representatives from Federal Agencies have participated in planning meetings on life science research at NSLS-II, including NIH staff from NIBIB, NCI, NIAID, NCRR, NIGMS and staff from DOE – BER and DOE – BES. Those able to attend this Workshop are listed here.

National Institutes of Health

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National Institute of Biomedical Imaging and Bioengineering
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C. Meeting Agenda

Thursday evening, June 4

7:45	Welcome & Introduction
8:00	NSLS-II Update/Overview – Steve Dierker
8:45	Characteristics of Insertion Devices & Front Ends – Qun Shen
9:30	Options/Considerations for Life Sciences Beamlines – Lisa Miller

Friday, June 5

8:30	Technical Discussion of IDs and Front Ends
10:00	Coffee Break
10:30	Management and Stewardship - Wayne Hendrickson
	Discussion of potential models for coordinated management of Life Science beamlines
2:30	Wrap up and Close