# NCRR/NIGMS NSLS II Working Group: Joint Study to examine opportunities for Life Sciences at NSLS II

The National Center for Research Resources and the National Institute of General Medical Sciences convened a working group on 27-28 April 2008, to advise us on the capabilities of and capacity needed for life sciences research at the National Synchrotron Light Source-II (NSLS-II). NSLS-II is a new Department of Energy synchrotron facility to be built over the next several years, replacing the current NSLS, and is scheduled to become fully operational in 2015.

## Panel members:

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## Executive Summary

Life sciences research at Brookhaven has been distinguished in equal measure by high-quality productivity as measured by the percentage of their publications that have been in very high impact journals, by innovation in areas such as coherent X-ray diffraction imaging and mail-in crystallography, and by educational outreach. These achievements are the more remarkable in light of the fact that NSLS is a second-generation synchrotron. The DOE decision to construct a third generation synchrotron to replace NSLS and terminate its operation presents a complex set of new opportunities for life sciences research and new questions about investment, implementation, and administration.

Construction of NSLS II poses two distinct challenges. The first is to assure continued access to technologies that will terminate with the deactivation of the NSLS synchrotron. This challenge must be met to sustain an exceptionally strong user group, primarily in macromolecular crystallography, and who are located chiefly in the northeastern United States. The second is to optimally exploit the physical characteristics of NSLS II in developing new, ground-breaking research facilities. It is desirable in

meeting both challenges to emphasize making all life sciences resources at NSLS II into truly national resources.

This panel was charged to consider each of these opportunities and address these challenges, by answering four questions about the status of synchrotron resources, new capabilities presented by NSLS II, needs and possible overlap and sharing of NSLS II facilities for macromolecular crystallography, X-ray scattering, Imaging and spectroscopy, and other techniques. It met 27-28 April 2008 in Bethesda to begin work on this charge, and its conclusions are contained in this document.

## Opportunities

Slightly less than half of NSLS users have been life sciences researchers. Of these, roughly 80 % have been macromolecular crystallographers. A similar profile is anticipated if resources are provided at NSLS II.

The NSLS II instrument will enhance opportunities for probing smaller crystals with even smaller beams than are currently available elsewhere. Although unquestionably important, the panel felt unanimously that although structural studies on small crystals could be done almost equally well at any third generation synchrotron, that the technical advantages at NSLS II will be more than incremental because the reduced beam dimensions will permit unique studies of X-ray damage surrounding the beam. This will permit the experimental investigation of relationships between the size of the irradiated area and the radiation damage suffered. Moreover, deactivation of the NSLS synchrotron represents a significant disruption for this community of users, which emphasizes the infrastructural aspects of the challenge: simply to sustain the existing level of access to instrumentation is an imperative. In this sense, if the NIH decides not to invest in infrastructure at NSLS II for macromolecular crystallography, it will be analogous to tearing down bridges on the Interstate highway system. Similar considerations apply to the various spectroscopic techniques planned for NSLS II, all of which stand to benefit incrementally from improved signal to noise ratios.

X-ray and spectromicroscopic imaging techniques will continue to form an integral part of the capacities on which life-sciences research depend. NSLS II plans to implement design decisions that afford scientific advantages for future research developments in this area. These advantages all stem from the very high brightness of the synchrotron radiation, and are realized in the increased coherence of better-defined beam geometries. This characteristic means that microscopic and coherent diffraction imaging technologies will benefit directly.

The most decisive of the qualitative advantages represented by NSLS II is in the area of solution scattering at both small and wider scattering angles, and in soft X-ray and coherent X-ray diffraction imaging. New computational methods are enhancing the amount and quality of information from solution scattering and these data are increasingly being used to constrain and/or validate results of molecular dynamics or normal mode analyses of protein structure and dynamics. These approaches provide unique insight into questions about the structure and behavior of macromolecules and macromolecular assemblies in solution. Consequently, they are being coordinated increasingly with crystallographic, NMR or computational studies of molecular structure and function and represent a particularly important probe for membrane structures which may be difficult to study by other methods. The panel concurred with the NSLS II white papers and presentations that bringing more photons into smaller cross sections will enhance time-resolved studies by reducing sampling times from tens of seconds to hundreds of microseconds. This change will bring biologically significant dynamical processes into an accessible range. Important synergies are evident in the joint application of scattering and other imaging techniques, including nanoprobe and X-ray footprinting, the latter having been a unique development at NSLS.

An important potential synergy recognized by the panel is that all of the spectroscopic, nanoprobe and footprinting methods can be expected to interact increasingly productively with the substantial enhancement of X-ray scattering at NSLS II, potentially enabling unprecedented levels of collaborative integration.

## Requirements

The advantages of higher brilliance at NSLS II mean that downstream beamline development will almost certainly require a new level of engineering for all, or nearly all, optical components in addition to state-of-the-art end-station hardware and especially environmental infrastructure with regards to isolation from vibrations. Whatever investment decisions ultimately evolve, the total amounts required will be very substantial. The NSLS II team presented a estimate of $200,000,000 in hardware costs to develop all of the proposed life sciences beamlines. The panel felt it unlikely that this amount would be forthcoming in a single investment, and that it might not even be appropriate. On the other hand, as noted below, discussion indicated that the considerable potential benefits of a comprehensive investment transcended the obvious one of obviating piece-meal implementation of life-sciences technologies at NSLS II.

Transitioning from NSLS to NSLS II presents challenges and opportunities from both scientific and management points of view. Indeed, NSLS II is catalyzing new thinking about how the NIH and the DOE can better coordinate stewardship of resources that have proven vital to the life sciences community. The NSLS II presentation team acknowledged that they had not completed their own assessment of how to meet these challenges, and the Panel consensus was that significant future work would be required from NSLS II representatives in cooperation with NIH and DOE to assure effective administration of life sciences at the new instrument.

## Conditional recommendations

The panel recommends unanimously that the X-ray diffraction capabilities of NSLS must be renewed, in order to sustain access to these facilities by the outstanding structural biology groups in the northeast. That support was not, however, unconditional. Two considerations are foremost. A significant majority of the panel argued that the use of three beamlines to serve conventional macromolecular crystallography projects, intended for installation with three-pole wigglers, was an unwise effort to recycle hardware from NSLS. As the costs of insertion device construction are comparable, the merits of recycling end-station hardware are offset almost entirely by the long-term consignment of these three beamlines to lower brightness applications. Thus, there was near consensus that the investment in protein crystallography should entail construction only of undulator beamlines that can remain state-of-the-art for the life of the instrument.

None of the panel members felt that the request for six undulator beamlines was justifiable in the short term. However, without the less bright 3-pole wiggler lines, the argument for a smaller number, perhaps 4 undulator beamlines was strong. This recommendation also matches the personnel (thirty individuals) now supported by various mechanisms (DOE BER and NIH P41) at NSLS to the stated goal of providing crystallography beamlines with 5-6 permanent staff. Implementing this recommendation, however, would pose the risk of a sustained interruption in operations during the NSLS – NSLS II transition, owing to the need to craft and test appropriate new optics.

The clear, cutting-edge match between the NSLS II instrument and small- and wide-angle scattering establishes a clear justification for the investment in beamlines for this application. Such investment generated perhaps the greatest enthusiasm. Scattering represents an area where methods development and application are proceeding rapidly and synergistically, and in ways that are entirely compatible with the technical design at NSLS II. Moreover, panel experts argued forcefully that scattering beamlines should

not be shared with other applications, as this substantially diluted their effective use. The panel endorses the implicit requests for 1.5 beamlines at the estimated costs proposed by NSLS II.

Spectroscopy appears to be an area where transitioning of existing technology is justifiable. Life sciences research will continue to rely on the resources that have developed at NSLS, so an argument should be made to sustain these technologies, even though they do not explicitly benefit from enhanced features of NSLS II. Moreover, with the exception of the X-ray fluorescence nanoprobe instrument, the costs associated with a single X-ray absorption and other spectroscopy installations are modest, compared with those associated with diffraction, scattering, and imaging applications.

Proposed imaging (and spectroscopy) applications were scrutinized somewhat less effectively by the panel, in part because, owing to an administrative breakdown, panelists who specialized in these fields were not transported to the meeting and had to interact over phone lines during the eleven hours of discussion. For this reason, specific recommendations are more difficult to formulate. The panel recommends continued development of these technologies at NSLS II. Enthusiasm will likely be greatest for undulator beamlines for coherent X-ray diffraction imaging scanning and soft X-ray microscopy, and Fourier transformed IR imaging and reduced for redundant facilities in X-ray absorption spectroscopy and scanning transmission X-ray microscopy for which the proposed capabilities are not significantly ahead of those at existing facilites.

X-ray footprinting is a unique product developed at NSLS. Although questions were raised, however, concerning the need for X-rays to generate the requisite hydroxyl radicals, a dedicated X-ray footprinting instrument on a damping wiggler beamline nevertheless was viewed with considerable enthusiasm.

Medical imaging and therapeutic applications are distinct from the expertise of the panel. The panel felt, moreover, that these were insufficiently supported by strong connections to a medical school. It thus recommends that support for these applications be evaluated and administered independently of support for research applications in life sciences.

## Unresolved Administrative Questions

All of these recommendations must be viewed as conditional on the development of a more mature proposal. In particular there is considerable concern that the NSLS II planning has not produced coherent prioritization, stewardship models, or implementation timetables. The NSLS II presenters were questioned intensely, and acknowledged that their planning process was not yet mature on these issues.

To a certain extent, the decision of the NIH to examine these questions in a coherent fashion before soliciting proposals represents an important opportunity to approach the investment, implementation, and management questions in an integrated fashion. Indeed, a sizeable and vocal part of the panel argued that the most successful models in the world – SSRL in the United States, and ESRF, Soleil, and Spring-8 around the world, are funded and administered in a coherent fashion that leads to significant economies of scale and important advantages in uniformity of service. Indeed, many of the life-sciences initiatives at NSLS – coordinated administration of several macromolecular crystallography beamlines, mail-in crystallography, and integration of these with biological spectroscopy and footprinting have emulated the administration at these other synchrotrons. Thus, although details of the administration of NSLS have been fragmented, with support from DOE BER, NIH P41, and the NY Structural Biology Center, it appears that much of their success in the life sciences research has resulted from decisions to coordinate the implementation of their diverse sources of support.

The biggest impact on life and translational sciences at the NSLS II would derive from a true programmatic priority and related management structure. Presenters hoped that day-to-day operations in life sciences at NSLS II could be integrated along the lines of the attractive “biology village” models at ESRF and elsewhere. A “Life Sciences Village” with appropriately dedicated resources could be a clear

differentiating factor and have real influence over the light source and report directly back to the main funding agency. In this regard, the decision by NSLS II to recruit a Life Sciences Director was viewed as a *sine qua non.* The panel felt it was of high priority to recruit the right person. While this recruitment should not preclude concurrent formulation of the overall development and management plan, the panel encourages the NSLS II leadership to task this director with development of the administrative and management planning necessary for an optimal implementation of such a "village".

In high-energy physics, it really was the synergy of co-location that drove innovation at that next level. The NSLS staff have developed considerable experience in such integration, as evidenced by the umbrellas of spectroscopy and imaging (Miller) and biophysical characterization (Sweet and Chance). For this reason, the NSLS II could benefit from having been "spring loaded" to begin operating in a more integrated fashion. A piece-meal mosaic of independently funded groups will not provide for a coherent effort. Proceeding on this basis, however, could raise investment costs, and will require considerable planning to resolve and/or prevent conflicts between funding agencies, and to implement a more than superficial collaboration envisioned by the "village" model. It is unfortunate that the NSLS II management has not recognized and begun planning how to accomplish this.

# I. Macromolecular Crystallography

Macromolecular crystallographic data collection represents by far the largest use of synchrotron radiation by the biological sciences. Roughly 40%-50% of users at US synchrotron facilities are in Life Sciences and 80% of the Life Science users that come to NSLS do so for X-ray crystallographic experiments. Additionally, macromolecular crystallography represents a significant fraction of the total and high profile publications from the scientific output of synchrotron facilities, with the majority of these publications coming from routine data collection. This high volume and high quality productivity makes decisions that affect the availability of any synchrotron for macromolecular crystallography critical to its total number of users and scientific output. Providing optimal resources for macromolecular crystallography is important both to NSLS II and its life science users in the regional and national context.

## A. Current capacity and capability of synchrotron resources for macromolecular crystallography

Current resources for macromolecular crystallography at US synchrotrons are reasonably matched to the demand for those resources. Exceptions include the lack of cost-effective, reliable service facilities at one end and ultra-small focused beams at the other end of the spectrum. The latter need is expected to be filled by beamlines at NSLS II. The panel expected that advances in technology such as faster detectors and automated sample changers will allow existing facilities to accommodate increasing demand if appropriately managed. However, the significant additional demand on other macromolecular crystallography beamlines resulting from loss of NSLS to its users without the replacement of its resources at NSLS II could not be easily met, highlighting the need at the former end of the spectrum. The questions the committee had to consider essentially come down to whether, or how much, demand will increase over the next decade, how much improved technology will reduce the time required by an individual user at what kind of facility, and how the unique capabilities of NSLS II will affect what research gets done there.

Already today more than two-thirds of data collection in macromolecular crystallography at SSRL is carried out remotely. Optimization of inventory and schedule management can readily realize a 24-48 hour turnaround for feedback cycles of routine screening and data collection. A substantial difference can really only be made for research programs local to the NSLS II where this feedback could happen in close to real time. However, there is a strong argument to be made that additional service facilities will be required at NSLS II to provide continuous access to synchrotron radiation in the context of both regional and national needs.

The primary conclusions of the panel were that it was essential, at a minimum, to replace the capacity and capability of the existing NSLS facilities, either by new facilities at NSLS II or improved facilities at other synchrotrons, that the planned beamlines should take advantage of the unique capabilities of NSLS II wherever possible, and that this must be viewed as part of a national plan not just a regional resource.

## B. Advantages of NSLS II

The facilities that are developed at NSLS II must take into account the unique capabilities of the NSLS II. However, for the majority of macromolecular crystallography as it is presently practiced, the high beam brightness that would be available at NSLS II would not appear to present an immediate benefit because it is not currently limiting at other synchrotrons. An emerging field that appears well-matched by the unique characteristics of the NSLS II is the increasingly routine data collection using 10 μm beam diameters and the expected benefits of smaller beam dimensions approaching 1 μm diameters. While this specification has been achieved at both the APS and SSRL, NSLS II will offer substantially brighter beams. Moreover, a newly constructed facility could more easily take the substantial design decisions to implement more vibration free facilities from the beginning. Anticipated improvements in much faster detectors will be well matched to very small, bright beams. Radiation damage will remain a limitation, and the much reduced beam cross-section represents the opportunity to study the physical location of damage, relative to the beam, and initial tests at APS suggest that there may be advantages to 1μm beams. Additionally, going back to multiple crystal data collection schemes is also possible.

Finally, NSLS II should not be regarded solely or even primarily as a regional resource. Nonetheless, it is obvious that requiring frequent access will drive researchers to look to nearby facilities. Such access to a state-of-the-art synchrotron facility is critical for successfully pursuing the most difficult problems in macromolecular crystallography, because sample preparation is nearly always the limiting factor and rapid feedback cycles between crystal characterization and sample preparation is critical.

## C. Number of beamlines needed for macromolecular crystallography

It is essential that at a minimum the existing NSLS data collection capacity be available at NSLS II. However, given the increased brightness of the beam and improvements that will be made in detectors and the routine use of automated sample changers, this will require fewer beamlines than presently exist at NSLS. Presently there are two undulator lines and 8 bending magnet lines with about 30 staff. If the NSLS II proposal of 5-6 staff per beamline were taken at face value, existing operations suggest 5 or 6 beamlines could be properly staffed with the current personnel resources. That number of undulator beamlines would seem to be an appropriate target to satisfy the eventual needs for both production and innovation. The panel felt that four undulator beamlines dedicated to macromolecular crystallography was justified from the outset.

There should be a clear separation between a macromolecular crystallography service operations that satisfy ~90% of the experiments and the experimental operations that attempt new approaches to using synchrotron radiation in macromolecular crystallography. The service operation needs to focus on reliable and cost-effective operation of a small number (2-3) of high performance, but not necessarily cutting edge undulator beamlines that are very well resourced. Such a service operation requires institutional and long-term support. A smaller number of more experimental beamlines could be funded in part by ‘the institution’ and in part by traditional models of funding, including individually targeted instrumentation proposals and/or industrial investors or along the lines of NSLS PRTs.

## D. Sharing beamlines with other techniques or scientific areas

As already noted, protein crystallography represents the majority of life sciences research at synchrotrons and the research requirements are best served by dedicated beamlines. The committee felt strongly that it was not reasonable to try to develop macromolecular service crystallography facilities on beamlines that were shared with other techniques. While it is possible to do so, the result would not be optimal for either

technique. Additionally, the potential beam time lost due to switching between techniques and reconfiguring equipment would be significant. Because the demand for beam time that is used for macromolecular crystallography is high, on a multifunctional beamline it would reduce the time available to other techniques.

There is, however, an important opportunity for co-location of different techniques as part of the technology development component. Beamlines common to multiple life sciences techniques would be well positioned to share resources. Simple examples are the ease of installing a crystallography detector on an imaging beamline. Although that should be trivial, it can be quite impractical if common software and hardware interfaces do not exist. This aspect is one of several examples highlighting the importance of integration and management, which the NSLS II presentation team unfortunately had not sufficiently addressed.

## E. Prioritization and staging of development

Many members of the committee thought that the plan to transition equipment currently located on the NSLS undulator lines to the NSLS II 3PW lines would not realize the savings suggested because the equipment would be somewhat outdated and additional technology developments may be required. In general, the undulator lines would not be significantly more expensive than the 3PW lines with all new, state-of-the-art equipment. Consequently, it was more reasonable to favor development of undulator lines.

A minority of the committee felt that providing a 3PW line for routine uses made good sense if it truly could be transitioned from NSLS at a significant saving. This would provide some distinction between the users needing capacity for high throughput experiments from the users that required a cutting edge beamline for particularly challenging experiments.

The timing of development of the crystallographic resources at NSLS II must be considered carefully. It is essential that the existing user community of NSLS be able to continue their excellent research programs with as little interruption as possible. On the other hand, it is equally clear that it is not reasonable to expect funding to develop six undulator lines and three 3PW lines simultaneously.
The committee felt strongly that the added capability of the undulator lines should give their development a much higher priority compared to the development of the three-pole wiggler lines that would increase capacity, but not provide the unique capability of the undulator lines.

## F. Process and Model; Conclusions

Several presentations made it clear that the macromolecular crystallography resources at NSLS II were viewed as providing a local or regional resource. However, the committee felt that if a large investment of NIH funds were to be made, it must be viewed as part of a national plan for synchrotron resources.

Nonfederal partners and users, especially industrial participation is considered to be desirable. However, the NSLS II will have to find a way to make investments in beamlines and equipment favorable to these communities. The committee did not see that having 20% of the beamtime reserved for such funding partners was likely to be a strong inducement.

The management approach to Life Sciences at the NSLS II was poorly thought out and hence poorly presented. Clearly, a coherent and consistent model would have to be put forward that would satisfy funding agencies/partners while truly serving the scientific community. Given the investment levels sought, that management has to be at the highest level of the NSLS. It is essential that the Life Sciences Director be appointed at the highest level of NSLS/BNL management.

NIH needs to re-inspect how it should fund and manage facilities and resources at NSLS II and coordinate its investment nationwide. Coordination between NIH, DOE and other possible present and future partners is essential to develop the required funding and management structure for macromolecular crystallography at NSLS II.

There clearly are problems to be solved with respect to funding and management models, priorities, and staging or phasing in the facilities. However, the NSLS II represents an important opportunity and the life science research community and funding agencies must take advantage of the opportunity.

# II. Scattering at NSLS II

Coordination of computational methods with solution scattering is enhancing the amount and quality of information that can be extracted from solution scattering patterns and these data are increasingly being used as constraints or tests of the results of molecular dynamics or normal mode analyses of protein structure and dynamics. These approaches provide unique insight into questions about the structure and function of macromolecules and macromolecular assemblies in solution. Consequently, they are being increasingly used in coordination with crystallographic, NMR or computational studies of molecular structure and function. They represent a particularly important probe for membrane structures which may be difficult to study by crystallography or NMR. Multi-protein complexes and signaling structures are specific examples of the kinds of structures that are well matched to the capabilities of solution scattering. The use of x-ray solution scattering - both small-angle (SAXS) and wide-angle (WAXS) - is growing rapidly, and in contrast to other life sciences applications, there likely will be a considerable unmet need for state of the art solution scattering facilities by the time NSLS II is in operation.

## A. Current Status

In the US there are 4 existing beamlines that devote a large fraction of their time for solution scattering. These include ALS 12.3.1; APS ID-18; CHESS G1; and SSRL-BL 4-2. Usage of these beam lines for solution scattering is rapidly increasing. Current facilities are adequate to provide most groups with beam time for proposed experiments at present, but we anticipate a substantial short-fall in the **near** future. By the time NSLS II is operational there will be a substantial shortage of beam time for these methodologies in the US.

## B New Capabilities

Beam characteristics for an undulator beam line at NSLS II appear to be ideal for solution scattering applications. There is every reason to believe that an undulator line at NSLS II outfitted with appropriate optics for solution scattering will be the best place on earth for solution scattering work. This is particularly true for time-resolved studies that will benefit from the high brightness of the undulator source. The combination of very small beam size and brightness will generate data of unprecedented quality and signal-to-noise ratio. Given the power of WAXS for discerning small changes in protein structure, these enhancements will be very important for many of the planned studies. High impact studies will include measurement of the amplitudes of normal modes of proteins in solution; the range of motion of enzymes undergoing catalysis; the measure of domain motions during substrate binding; and the progression of structure formation during protein folding.

## C. Recommended number of beam lines at NSLS II

Our recommendation is that NSLS II develop **two beamlines** for solution scattering applications. These should include an undulator beam line optimized for time resolved studies and a 3 pole wiggler beam line for static studies. The use of these facilities should be limited to scattering studies of solutions of macromolecules; fibrous specimens and membranes. Specimen handling equipment for automated aspiration of samples from 96 well plates should be provided. Additional equipment for automatic mounting of fiber and/or membrane specimens should also be available. Capabilities for using a very broad range of specimen-to-detector distances should be available, making possible collection of WAXS data to at least 2 Å spacing, and SAXS data to very small angles. A very high speed two-dimensional detector (e.g. a PILATUS detector with at least 2k x 2k elements) should be available for use on the

undulator beam line. A second detector with at least 2k x 2k elements should be available on the 3-pole wiggler beam line.

### D. Feasibility of sharing with other techniques or with material scientists

Beam lines for solution scattering previously have been shared with other techniques. **We would strongly discourage** development of beamlines intended to be split between multiple techniques such as SAXS/WAXS and spectroscopy (e.g. XAS). Users who have collected data at multi-purpose beamlines have routinely encountered excessive setup time and been forced to collect data with a beam that has not been adequately optimized for the particular application. Beamlines dedicated to a single technique are much more likely to be run with optimized parameters and require little if any set up time.

In many cases it will be possible to use these beamlines for fiber diffraction studies with essentially no change in the hardware or alteration in the beam parameters. For the most part fiber diffraction experiments require collection of data to scattering angles comparable to those used in WAXS studies. Studies of membrane specimens, including those exhibiting two-dimensional order in the plane of the membrane usually require optics comparable to that used for fiber diffraction. In some cases, the work is limited to scattering at smaller angles, and it is likely that the SAXS cameras will be close to the optimal configuration required.

Studies of soft (non-biological) materials that require essentially identical optics represent appropriate use of these beam lines as long as the optics required are consistent with those used in biological studies. Specimen handling may be very different for non-biological specimens and applications that use specimen holders that require substantial re-configuration of the beamlines should be declined.

## III. Spectroscopy

Biological X-ray absorption spectroscopy (XAS) is a relatively routine technique that provides element-specific electronic and local molecular structural information about trace elements (e.g., metals) in biological samples. It helps provide the chemical speciation associated with other techniques (e.g., microprobe imaging, discussed elsewhere) and can be applied to single crystals to obtain polarized structural information. The latter application represents a small but growing recognition of the utility of performing both x-ray spectroscopy and x-ray diffraction on single crystals of metalloproteins. X-ray spectroscopy provides both electronic and molecular structural information, and can provide an important *in-situ* monitor of radiation damage during MX data collection. NSLS II should consider the potential of providing this feature on one or more of the MX beam lines, along with other microspectrophotometric techniques (using non-synchrotron sources, such as visible or IR).

Biological XAS is more usually applied to (frozen) solution or biological specimens. Generally speaking, current biological XAS beam lines are not flux-limited for standard samples; they are more often detector-limited. Thus, the increased brightness at NSLS II will have little effect on the kinds of experiments that can be performed. Exceptions would be high-throughput screening in which low-volume samples and continuous rapid-scanning monochromators are employed. These applications will also benefit from automation of sample changing and data collection, which is slightly more involved than that employed for automated crystallographic screening, but should be pursued.

### A. Current Status

Currently, several US beam lines are dedicated to, or heavily used for biological XAS, and these beam lines essentially meet the existing demand for standard experiments.

## B New Capabilities

Consequently, new sources have been reluctant to dedicate beam lines to XAS. It is important to note that the use of this technique is essentially self-limiting given the need for a biochemist to collaborate with a biological XAS expert. On the other hand, if this technique were to become more user-friendly, the number of non-expert users would increase. This growth in demand might be met by implementing more automated sample changing and data collection procedures on any of these existing beam lines, and these steps may even lead to "mail-in" operation, if done properly.

## C. Recommended number of beam lines at NSLS II

Areas in which NSLS II could consider contributing to XAS might include improved sample automation, continuous-scan monochromator development, and detector development. Improved detectors that provide both larger areas of coverage for fluorescence detection and higher energy resolution, combined with high throughput, are sorely needed. With these developments, current NSLS and future nationwide demand for XAS beam time might support one new NSLS II biological (3-pole wiggler-based) beam line. It will be necessary to develop rapid scanning methods to ameliorate radiation damage when exploiting the full brightness expected at NSLS II. Note that most of these activities are essentially source-independent, and could be pursued at many of the XAS beam lines found in other facilities.

### UV Circular dichroism

The current bending magnet beamline on the NSLS UV ring (U11) is proposed to move to NSLS II and transitioned to a bending magnet with 75% of the proposed time used for life sciences research. At NSLS the structural biology community has been the main user of CD, though this usage remains fairly small. The beamline would have a large horizontal acceptance and a two-element monochromator. End station equipment for stopped-flow and for the application of magnetic field are proposed. With beamlines for CD at other synchrotrons, this is not a high priority, although there is excellent local expertise in this area.

# IV. Imaging

In the next few years, there is likely to be an increasing demand for synchrotron beamlines dedicated to x-ray imaging and microscopy. Some of the capabilities that will be needed include real-time imaging of physiological processes, high-resolution anatomical imaging of organisms, imaging whole eukaryotic cells at a nanoscale, and mapping trace concentrations of chemical elements within cellular organelles. X-ray imaging and microscopy offer unique advantages for obtaining these types of information.

Unlike macromolecular crystallography, however, x-ray imaging and microscopy requires a considerable amount of further development before the techniques can be routinely applied to address important biomedical questions. For example, it is necessary to provide fast acquisition of 3D tomography data from well-preserved frozen-hydrated cells, to enable higher throughput nanoscale elemental mapping by increasing photon flux and detector efficiency. Considerable additional funding will be required to develop and apply these capabilities for biomedical research.

### X-ray Fluorescence Microscopy (XRF):

Current XRF micro/nanoprobe beam lines typically produce images at relatively slow rates (e.g., several hours for acquisition of 200 x 200 pixels). Such data rates currently preclude analysis of large numbers of cells required for many biological studies. It is unclear to what extent data rates can be increased in third-generation synchrotron sources, although the low emittance of NSLS II promises to provide higher spatial resolution: increased detector solid-angle will not necessarily provide improved detection limits due to the dependence of the background on polarization direction. On the other hand, it might be feasible to perform 3D elemental imaging using tomographic techniques by using the principle of dose fractionation to record low-dose tilt series, which can be reconstructed provided fiducial makers can be used for alignment.

Three XRF micro/nanoprobe beamlines are proposed, including one dedicated undulator BL with intermediate spatial resolution (50-1000 nm) shared with environmental science, one undulator BL with high spatial resolution (<50 nm), and a 3-pole wiggler BL operating at micrometer resolution and shared with environmental science. This represents a significant expansion of effort in this area relative to NSLS's one XRF microprobe BL, which is shared 50% with environmental sciences. The need for three XRF micro/nanoprobe BLs seems to be largely driven by the slow data acquisition rates. To justify the additional beamlines, it is important to provide new capabilities such as development of higher throughput, cryo, and tomographic acquisition modes.

**Transmission (TXM), Scanning Transmission (STXM) X-ray microscopy and Coherent Diffraction Imaging (CDI):**
X-ray microscopy offers the potential for imaging whole eukaryotic cells with thicknesses up to 50 micrometers and complements optical and electron microscopy. However, because of the rapidly expanding number of alternative imaging techniques based on optical microscopy, which can be applied to living cells and which can provide information about specific molecular distributions, a stronger case is needed about what important biomedical problems can be specifically addressed by x-ray microscopy.

Three different types of instrument are available for x-ray microscopy, and it is proposed to develop beamlines for each: scanning soft x-ray microscope (STXM) undulator BL, STXM 3-pole wiggler BL, transmission x-ray microscope (TXM) bending magnet BL, coherent diffraction imaging (CDI) undulator BL. Many of the ideas underlying STXM and CDI were developed at NSLS and the strong in-house expertise makes this research very appropriate for NSLS II.

It is essential to develop x-ray microscopy into a three-dimensional imaging technique that can be applied to frozen-hydrated specimens (as has already been accomplished by C. Larabell et al. at ALS). To achieve this 3D capability for STXM, CDI as well as TXM is a *sine qua non* that will require considerable effort and funding.

It is also not clear that three beamlines can be justified given that x-ray microscopy has not yet answered any major biomedical research questions. Clearly considerable attention must be given to what class of problem can be solved by these techniques.

The sharing of all types of x-ray microscopy beamlines with environmental or materials science seems to be reasonable but would not necessarily be consistent with the biology village concept.

**Infrared Imaging:**
NSLS has the world's largest effort in synchrotron-based Fourier Transform Infrared Microscopy (FTIRM), which are producing a steady stream of research publications. Three bending magnet beamlines are currently allocated to FTIRM at NSLS, and it is proposed to move all three to NSLS II. It is projected that these FTIRM instruments will operate on NSLS II without loss of performance. The panel felt that this is an important program and an excellent approach.

Many applications of FTIRM require the use of complementary x-ray imaging techniques. For example, FTIRM can identify plaques of misfolded amyloid beta peptide in Alzheimer's disease brain, which can then be analyzed by XRF microprobe to determine potential co-localization with trace metals. Having multiple techniques available in a dedicated biology village environment would make such experiments easier to accomplish even though they would have to be performed on two separate beamlines.

**Medical Imaging and Radiation Therapy:**
Many pioneering developments in medical imaging occurred at NSLS, which included development of diffraction enhanced imaging (DEI) as well as high-resolution CT coronary angiography, and the use of collimated x-rays for radiation therapy. It is proposed to move this effort to a superconducting wiggler beamline, which will produce a high flux of high-energy (20 keV) x-rays. The development of a long

beamline can be expected to provide new capabilities for dynamic imaging of small and medium-sized animals at high spatial resolution. The eventual extension of this beamline for human use is less certain and would depend on DOE policy and on forging strong links with one or more regional medical centers, which has not yet been encouraged by NSLS leadership. For this reason, and because the panel had limited expertise in medical imaging and therapeutic applications, our recommendation is that a more specifically targeted review would be appropriate for such resources.

## V. Other - X-ray footprinting

The use of intense white x-ray beams to create hydroxyl radicals that cleave the backbone of nucleic acids and modify protein side chains, when used in conjunction with gel electrophoresis for nucleic acids and mass spectroscopy for proteins, can map regions of solvent accessibility and thus provide information on the tertiary structure of these biomolecules and their assemblies in solution. This method, referred to as X-ray footprinting, is a technique that currently is practiced almost exclusively on bending magnet beamline X28C at NSLS (although nascent efforts at ESRF and LNLS are ongoing). This is a facility run by the Center for Synchrotron Biosciences (CSB) of Case Western University under the direction of Mark Chance. Scientific results obtained using this method as a step in the procedure, has led to information on the time-dependent folding of RNA in the Tetrahymena ribozyme, and maps of the interfaces of protein-protein interactions. The CSB interacts strongly with BNL and NSLS, and it is expected that this program be well integrated with other life sciences beamlines at NSLS II. Using this method at X28C there have been nine publications in 2007, six in 2006, thirteen in 2005, and twelve in 2004. Almost all of these featured Dr. Chance as a principal or as a collaborator. A strength of the existing program is the suite of multi-technique beamlines that it develops and operates at NSLS, in a manner that is designed to promote multi-method studies of biological molecules. In this regard a straightforward footprinting beamline would fit well into the biology village concept.

The beamline optics are straightforward particularly since these experiments use the "white" beam, with a focusing mirror and apertures in the current bending magnet beamline. At NSLS II, it is proposed that an end station of a damping wiggler be used for these experiments. A vertically-collimating mirror will accept 1.0 milliradian of horizontal radiation from the wiggler and deliver white beam on the sample. Some of the instrumentation from the current bend magnet end station can be transitioned to NSLS II, and end station instrumentation for automated sample handling and ultra-rapid mixing are planned. Footprinting at higher time resolutions will be achievable with the high-flux from this source. The shorter time exposures will improve the data for both stationary and time-resolved studies. Several interesting experiments that are planned to be addressed by this method on GPCRs, and macromolecular assemblies would all have a positive impact. The future level of usage, and thus needed capacity, of this method are difficult to estimate, except that it should increase. As this is a simple beamline on a damping wiggler, and a well-established program of scientific and technical collaborations are already in place, this beamline would be worthwhile to support. As the CSB currently collaborates with, but is not integrated into, NSLS and BNL, it is important that the model of facility-run beamlines is funded, developed, and managed in a manner that is consistent and realistic.

## VI. Institutional Issues

A primary issue is how life sciences beamlines at the NSLS II will be funded, developed, and managed. The PRT/CAT models used at the NSLS and APS are widely acknowledged to have both benefits and faults. On the plus side, they harness a great deal of community involvement, support, and innovation. On the negative side, many such efforts have had difficulty maintaining these three key resources as the original participants moved among institutions and changed scientific interests. There is also the sense that in many cases the general user community was not being adequately served.

DOE BES responded by moving to a primarily facility owned and managed beamline model. Historically, this has worked well at some sources (e.g., SSRL, ALS, CHESS). But each source is different and has to develop solutions tailored to its specific circumstances. What might work well at the ALS, where there is close physical proximity to Berkeley, might not work at the NSLS II. Beamline costs at the NSLS II will be high – on order of $10M each – if the source is to be utilized to full potential and many non-BES beamlines will need to be developed. The committee is skeptical that a one-size-fits-all model will be successful.

Broadly speaking, life sciences beamlines at the NSLS II are of two types. The first are state-of-the-art beamlines that the community already knows how to build. This includes most of the core macromolecular crystallography beamlines. Although it takes a tremendous amount of effort to develop these beamlines, there are well-established beamlines elsewhere that can be emulated. In general, these beamlines are not dependent on source attributes solely specific to the NSLS II. These types of beamlines can be readily developed by appropriate BNL staff, assuming that enough experienced personnel can be found (which will be a challenge). Much of the work can be contracted to industry. These types of beamlines can relatively quickly commissioned after assembly to serve a very wide user community. In this case, there is reasonable confidence that competent BNL staff can build the beamlines. Here, institutional issues center on how beamline construction and operation will be funded and managed. NIH would reasonably demand some measure of control throughout this process if it is to foot the bill for construction and operation. However, it is unclear what level of such control would be optimal in return for its investments.

The second type of beamline exploits new capabilities (e.g., coherence) beyond what the community already knows how to build. These beamlines are really R&D projects that will, no doubt, take years to develop, and will certainly benefit from a deeper engagement of the broader university community interested in x-ray technology. Development of these beamlines will require years of climbing a steep learning curve, both with respect to construction and to experimental utilization. The user community barely exists for these types of beamlines, not because of lack of potential, but because these types of beamlines do not yet exist. Historically, the user community develops based on case examples of frontier x-ray science by the people committed to doing the beamline technology R&D. This is a slow process. It is unrealistic to expect this type of beamline to be quickly commissioned and turned over to general service, yet these are the beamlines that will most truly utilize unique attributes of the NSLS II source, and therefore, most justify the extra expense of building a source beyond that of a Diamond or a Soleil. Here, institutional issues center on how to provide sufficient incentives to engage the university community for the many years of gestation required.

The committee was hoping to hear an analysis of a beamline development and operation model suitable to the nuances of the NSLS II. Unfortunately, all that was presented was a set of general constraints, to the effect that NIH beamlines would be built by BNL staff, facility owned, and quickly turned over to 80% general user time. It was unclear as to the role NIH would play in critical decisions about the construction and continued management of the beamlines. There was no discussion of the R&D process for beamlines beyond the state-of-the-art and how adequate incentives would be provided to engage university scientists for years of effort. The village concept was presented. It has potential, in principle, but the level of detail provided about exactly how it would work –how critical decisions would be made, who would bear responsibility for mismanagement, how costs would be shared, etc. -- failed to rise above that of sound-bites, and was therefore disappointing.

The committee recognizes that the NSLS II is in an early stage of development. However, engagement of non-BES partners cannot proceed without a better definition of appropriate beamline development and operational models. The committee strongly encourages the NSLS II management to initiate this discussion with potential partners as soon as possible. Flexibility and compromise will be required.