NSLS & NSLS-II SCIENTIFIC STRATEGIC PLANNING WORKSHOP SUMMARY

LIFE SCIENCES

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LIFE SCIENCES OVERVIEW

Introduction

Life science users represent ~45% of the user community at NSLS. They can be broadly categorized into two research communities: structural biology and chemical/structural imaging. The former community focuses its research on the determination of the atomic structure, dynamics, and function of biological molecules and complexes using synchrotron techniques such as macromolecular crystallography (MX), small- and wide-angle x-ray scattering (SAXS/WAXS), x-ray absorption spectroscopy (XAS), x-ray footprinting, and UV circular dichroism (CD). The imaging community's research typically involves understanding the structural and chemical composition of biological materials, such as cells and tissues, at a wide range of size scales from <50 nm to >1 mm. Synchrotron-based imaging techniques include scanning transmission x-ray spectromicroscopy (STXM), full-field transmission x-ray microscopy (TXM), coherent diffraction imaging (CDI), computed micro-tomography (micro-CT), x-ray fluorescence microscopy (XFM), Fourier transform infrared microspectroscopy (FTIRM), and diffraction-enhanced imaging (DEI).

The body of this document contains whitepapers prepared by different disciplines of the Life Sciences community that participated in a Scientific Strategic Planning Workshop held on January 15-16, 2008. They describe current programs that are thriving at NSLS today, proposals for short-term improvements to current facilities, and proposed programs and infrastructure for the future at NSLS-II. While each whitepaper describes proposals for specific experimental programs, it is most important to emphasize that the overarching conclusion from the workshop was the desire within the life-sciences community to see increased interaction, collaboration, multi-technique integration, and cross-disciplinary approaches to doing science in the future. Workshop participants used the term "Biology Village" to denote this mode of research and support establishment of such an environment at NSLS-II. The Life Science community would prefer that beamlines predominantly involved in serving the Life Science community, as well as those serving related communities, such as environmental sciences and soft matter biophysics, be located as near to one another as possible, subject to the other constraints that the facility must take into consideration in assigning beamline locations. In addition to support space in the Laboratory-Office Buildings (LOBs) for Life Science beamlines, a need is also foreseen for a separate, centralized facility for Synchrotron Structural Biology and Imaging at NSLS-II to provide capabilities that go beyond what can be accommodated in the LOBs.

As a preface to the detailed white papers from each discipline, a brief survey of the current state of lifescience at NSLS is presented, followed by a summary of the beamlines proposed for NSLS-II in the whitepapers. Along with this we can mention obvious needs for R&D to accomplish this, describe the infrastructure required on the NSLS-II floor or in the LOB, and will summarize the synergy we expect between life science and the other disciplines at NSLS-II.

NSLS Life Sciences Programs Today

The **MX** (macromolecular crystallography) user community represents approximately 80% of the life sciences users at NSLS. Today, NSLS has 10 macromolecular crystallography beamlines – eight on bending magnets (X3A, X4A, X4C, X6A, X8C, X12B, X12C, and X26C) and two on undulators (X25, X29). In general, the capacity for the bending magnet beamlines meets the user demand; both undulator beamlines are oversubscribed.

SAXS/WAXS (small- and wide-angle x-ray scattering) are growing in popularity among the structural biology community, employed to study the structures of proteins in solution and "solubilized" membrane proteins, large macromolecular complexes, and system dynamics. NSLS currently has one SAXS beamline (X21), but this program is woefully oversubscribed because it is shared with resonant and magnetic scattering programs. However, beamline X9, which is currently under construction and slated for completion in the summer of 2008, will be fully dedicated to SAXS and WAXS for both life sciences and soft condensed matter applications.

XAS (x-ray absorption spectroscopy) is perhaps the most widely used technique at NSLS among many research communities. While life science users can use any of the XAS beamlines, there is only one beamline dedicated and specifically equipped for life sciences applications (X3B). Beamline X3B is consistently oversubscribed and near-term plans include the purchase of an upgraded Ge detector to enhance detection sensitivity and data collection rates. To meet the scientific needs, the general user time will be increased to 75%.

Beamline X28C is currently the only facility in the world fully dedicated to **x-ray footprinting**. Owing to growing demand for the technique, other synchrotron facilities (ESRF, LNLS) have recently developed beamline facilities that support x-ray footprinting. Since x-ray footprinting data are acquired quickly, this beamline is not fully subscribed and could accommodate a five-fold increase in users (with additional staffing).

At present, two beamlines at NSLS are devoted to **UV circular dichroism** and related spectroscopies (U9B, U11). Upgrades are planned for U11, which has superior VUV penetration, and include a new monochromator, improved sample chamber, new beamline computer system, improved polarization optics, and the possibility of a multiwavelength detector.

NSLS has the largest **infrared program** worldwide, with 6 active beamlines and 4 infrared microscopes. Two IR microscopes are currently used for life sciences applications (U2B, U10B), and both programs are oversubscribed. In order to address the high demand – especially for life sciences users – a new infrared imaging microscope was recently funded by the NIH and is being commissioned at beamline U4IR.

STXM (scanning transmission x-ray spectromicroscopy) was pioneered at NSLS and has now spread to a number of facilities worldwide. The present-day STXM program at NSLS is housed at undulator beamlines X1A1 and X1A2. This user program serves the life, environmental, and soft condensed matter communities and is fully subscribed. The first cryo-STXM for frozen hydrated samples was also operated at NSLS, where it received an R&D 100 award in 1999, though that microscope has since been taken offline while its successor is planned.

Micro-CT (computed micro-tomography) is performed on beamline X2B. While this program primarily serves the materials and earth sciences communities, the life sciences

community is growing. Nanotomographic methods do not currently exist at NSLS, although both the life sciences and materials science users have shown an interest in developing full-field x-ray nanoCT, where current instruments at NSRRC (Taiwan) and SSRL (Stanford, USA) have demonstrated a spatial resolution of 30 – 50 nm.

Medical imaging and **radiation therapy** have a long tradition at NSLS superconducting wiggler beamline X17B. In fact, three of the five major medical research applications of synchrotron were initiated and developed at NSLS. These include the microbeam radiation therapy (MRT) and the photon activation therapy (PAT) programs, which operate at X17B1, and the diffraction enhanced imaging (DEI) program, which runs at X15A.

CURRENT LIFE SCIENCES BEAMLINES AT NSLS

Number	Beamline	Technique	Source	PRT/FB	% life sciences	shared with…
1	X3A	MX	bend	PRT-Case	100%	
2	X4A	MX	bend	PRT-NYSBC	100%	
3	X4C	MX	bend	PRT-NYSBC	100%	
4	X6A	MX	bend	FB	100%	
5	X8C	MX	bend	PRT-PXRR	100%	
6	X12B	MX	bend	PRT-PXRR	100%	
7	X12C	MX	bend	PRT-PXRR	100%	
8	X25	MX	undulator	PRT-PXRR	100%	
9	X26C	MX	bend	PRT-PXRR	100%	
10	X29	MX	undulator	PRT-PXRR	100%	
11	X21/X9	SAXS/WAXS	undulator	FB	50%	soft materials
12	X3B	XAS	bend	PRT-Case	100%	
13	X28C	x-ray footprinting	bend	PRT-Case	100%	
14	U11	CD	bend	PRT-BNL Bio	100%	
15	U2B	FTIRM	bend	PRT-Case	100%	
16	U10B	FTIRM	bend	FB	50%	enviro, soft materials
17	U4IR	FTIRI	bend	FB	75%	enviro
18	X27A	XRF microprobe	bend	FB	50%	enviro
19	X1A	STXM	undulator	PRT-SBU	35%	enviro, soft materials
20	X2B	MicroCT	bend	PRT-ExxonMobil	25%	geosciences, materials
21	X15A	DEI	bend	FB	100%	
22	X17B1	MRT	SCW	FB	25%	geosciences

Proposed Life Science Beamlines at NSLS-II

The **MX** community believes that six undulator beamlines and three three-pole wiggler (3PW) beamlines are needed for MX at NSLS-II. The undulator beamlines would prefer low-β straight sections, and the six beamlines could likely be served by just three straight sections by using canted undulators to yield two beamlines per straight section. At least one undulator beamlines should be devoted to high-throughput measurements and at least one should be instrumented to handle the most challenging problems and to allow the use of ancillary techniques. The three beamlines built using 3PW sources should be conventional MX beamlines. These might be constructed from usable components of the existing ten NSLS MX beamlines.

In **x-ray scattering**, life-science research encompasses a large variety of samples and different variations of experimental methods. Therefore, the community believes that three different x-ray scattering beamlines are required, at the minimum, to meet the anticipated research needs: (1) a 3PW beamline dedicated to solution scattering to meet the increasing demand for a fully dedicated, high-efficiency solution scattering instrument (2) an undulator beamline for high-brightness measurements such as time-resolved studies, two-dimensional membrane structure, and hierarchical structure in tissues, and (3) a reconfigurable 3PW beamline for measurements such as fiber diffraction and model membrane diffraction on bulk samples, which are best carried out at a beamline that can be easily reconfigured for different measurements. For both the undulator beamline and the reconfigurable 3PW beamline, the soft condensed matter community is likely to have similar needs and could share these beamlines.

For **bio-XAS**, the community proposes that beamline X3B transition to a 3PW at NSLS-II and make full use of the beam time. Taking into consideration the expected user demand at the primary Bio-XAS facility, the life sciences community suggests that a second 3-pole wiggler XAS beamline be shared with the overlapping field of earth and environmental sciences.

X-ray footprinting applications require a stable, broad energy range, high flux source. A 3.5 m canted section of an NSLS-II Damping Wiggler (DW) is an ideal source for this purpose. Thus, the community proposes that beamline X28C transition over to a DW source, enabling x-ray footprinting experiments to reach much shorter exposure times and investigate previously unapproachable systems.

For **UV-CD**, the community proposes that beamline U11 transition to NSLS-II. It would need the largest possible horizontal acceptance, which would be a large-gap IR bending magnet port.

The community proposes that the vibrant **infrared microspectroscopy and imaging** programs that currently operate at NSLS transition to NSLS-II. For the life sciences community, it is proposed that there be two confocal microprobe endstations and one full-field infrared imaging endstation, all built on standard-gap IR bending magnet ports.

PROPOSED LIFE SCIENCE BEAMLINES AT NSLS-II

Number	Technique	Source	New or Transitioned?	Potential Transitioned NSLS Beamline	% Life Sciences	Shared with…
1	MX	undulator	new		100%	
2	MX	undulator	new		100%	
3	MX	undulator	new		100%	
4	MX	undulator	new		100%	
5	MX	undulator	new		100%	
6	MX	undulator	new		100%	
7	MX	3PW	transitioned	X6A	100%	
8	MX	3PW	transitioned	X25	100%	
9	MX	3PW	transitioned	X29	100%	
10	SAXS/WAXS	undulator	transitioned	X9	50%	soft materials
11	SAXS/WAXS	3PW	new		100%	
12	SAXS/WAXS	3PW	new		50%	soft materials
13	XAS	3PW	transitioned	X3B	100%	
14	XAS	3PW	new		50%	enviro
15	x-ray footprinting	DW	transitioned	X28C	100%	
16	CD	bend	transitioned	U11	75%	materials
17	FTIRM	bend	transitioned	U10B	50%	enviro, soft materials
18	FTIRM	bend	transitioned	U10A	25%	enviro, soft materials
19	FTIRI	bend	transitioned	U4IR	50%	enviro, soft materials
20	XRF microprobe	3PW	transitioned	X27A	50%	enviro
21	XRF sub-microprobe	undulator	new		50%	enviro
22	XRF sub-microprobe	undulator	new		100%	
23	TXM	bend	new		50%	materials
24	STXM	undulator	new		25%	enviro, materials
25	STXM	3PW	transitioned	X1A	35%	enviro, soft materials
26	CDI	undulator	new		50%	
27	DEI, MRT	SCW	transitioned	X15A, X17B1	100%	

The community expects the **X-ray fluorescence microscopy** programs at NSLS-II to benefit greatly from the very high brightness of the undulator sources. A canted pair of XRF undulator beamlines and a 3PW beamline are proposed to serve the life and environmental sciences communities. One undulator beamline should be based on KB focusing optics (high flux, broad spectral range, 1 μm spatial resolution) and the second undulator beamline should be based on

zone plate focusing optics for high spatial resolution (50 nm or better). The 3PW beamline might be constructed from usable components from the existing NSLS beamline X27A.

The community proposes a dedicated **medical imaging and therapy** beamline employing a superconducting wiggler. The beamline design should allow future advancements to studies with large animals and eventually humans. It is proposed that the experimental hutches have adequate length and width to allow the irradiation hutch, together with supportive hutches, to form a combined medical suite.

The high brightness of NSLS-II will make it possible to tightly focus the beam to create very intense **nanoprobes** for high-resolution subcellular imaging and sensitive trace element mapping in biological specimens. The brightness will also provide highly collimated beams of high intensity and large transverse dimensions for novel forms of medical imaging and tomography. Thus, the community proposes that the **soft x-ray spectromicroscopy** program at NSLS-II be served by an undulator beamline and be a 3PW beamline, with cryogenic sampling and nanotomography capabilities. The 3PW beamline might be constructed from usable components from the existing NSLS beamline X1A.

In addition, a number of imaging techniques that are not currently available at NSLS are proposed for NSLS-II, including **coherent diffraction imaging (CDI)** and **full-field x-ray nanotomography (nanoTXM)**. A CDI beamline is proposed, with one endstation intended for biological imaging at energies as low as 2.5 keV. A nanoTXM beamline is also proposed for NSLS-II. Thus, combined with current techniques that will be transitioned from NSLS, these diverse imaging tools will span the resolution scale from nanometers to millimeters, allowing non-destructive analysis of biological subjects ranging from sub-cellular structures to humans.

R&D Needs

A number of research and development needs are necessary for successful implementation of the new programs at NSLS-II. Each chapter specifically describes the technique's individual needs, but most indicate a strong emphasis in optics and detector development, sub-micron sample positioning and stabilization, cryo specimen handling, and data analysis and storage.

Facility Infrastructure at NSLS-II

NSLS-II presents a unique opportunity to concentrate complementary techniques and expertise in a single location to provide a comprehensive life science capability (Figure 1). The main purpose is to provide state-of-the-art research tools to the Life Science Community. We believe that extensive ancillary facilities are needed for the research at NSLS-II to reach its full potential. To ensure the success and research productivity, it will be crucial that these laboratory facilities be adequately staffed to provide technical and scientific support to the user community and to assist staff scientists in their own research.

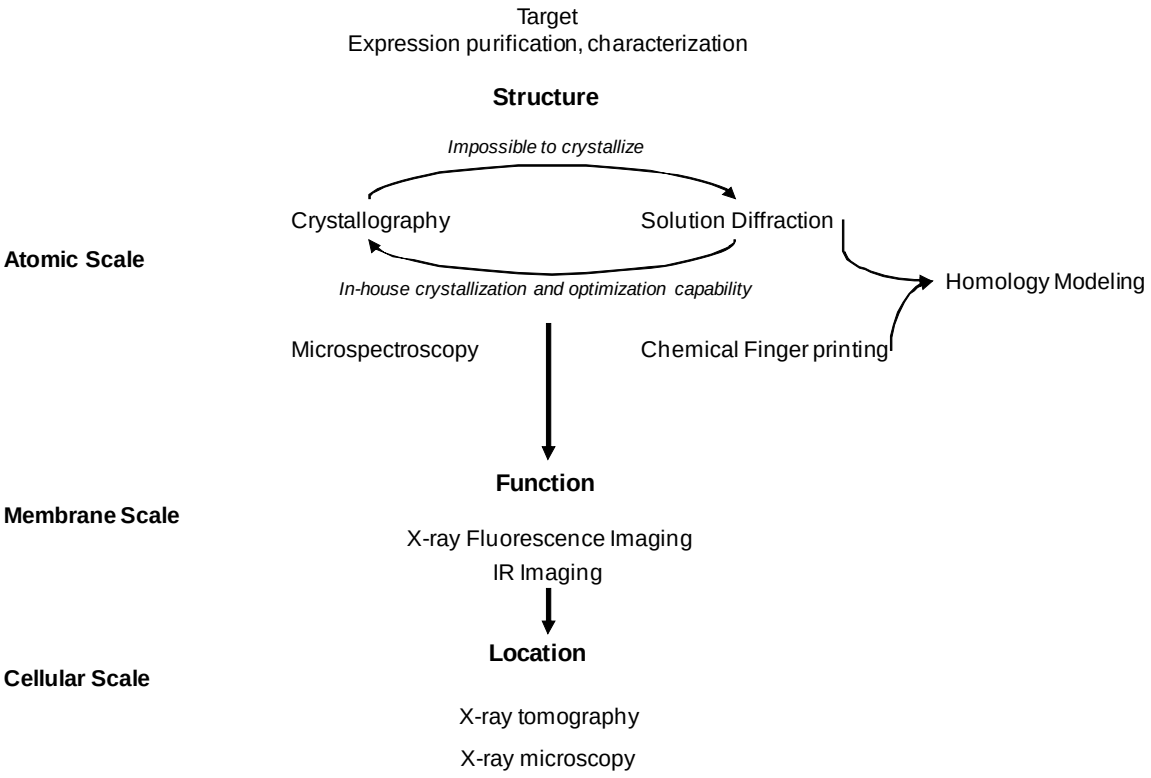

Figure 1. A 'straw man' concept for an integrated life science structural process. (from E. Snell, Hauptman-Woodward Medical Research Institute; Life Science Workshop, Jan 15-16, 2008).

LABORATORY NEEDS of the community can be divided into three main areas:

1. **Chemistry and biology labs**: the success of many techniques in life science is intimately related to sample quality. While specific needs are described in the following sections, general requests include instrumentation and techniques that allow the production, purification, and characterization of proteins. Microscopy research is highly dependent on sample preparation and one must often interleave variations in preparation protocols with microscope observations in order to arrive at a good protocol. Shared core facilities, such as cold rooms, crystallization rooms, centrifuge rooms, liquid chromatography, mass spectroscopy, dynamic light scattering, UV-visible spectroscopy, FTIR spectroscopy, CD spectroscopy, and environmentally controlled sample preparation and storage areas are needed. The community requests that sample preparation labs for life science research include a Biosafety Level 2 (BSL-2) wet lab with cell culture facilities, centrifuge, refrigerator, freezer, and sterile laminar flow hood. This lab should also include inverted and confocal light microscopes with fluorescence and phase contrast capabilities. Because cryo-vitrification methods will grow in importance as the spatial resolution of x-ray nanoprobes and microscopes is improved, a cryo-prep lab with plunge and high-pressure freezers, freeze dryers and freeze substitution unit, cryo-ultramicrotome, cryo-light

microscope, and liquid nitrogen storage would be needed (perhaps supplemented by a laboratory x-ray source for evaluating the presence or lack of ice diffraction rings). These labs should be staffed both to assist users with correlative techniques and to make sure that lab equipment is well treated and remains in good service.

2. **Instrumentation labs**: for ongoing beamline upgrades and development; these may include an area for a machine shop and electronic lab; staging areas for end-station instrumentation storage.
3. **Computer labs**: offering central data storage capability and development and maintenance of essential software for real-time data processing. A computer lab will also be needed for data analysis and processing beyond what is done in real time at individual beamlines.

Not included in this list are the needs of the Medical Imaging & Radiation Therapy community, which requires extensive animal facilities and associated equipment and support.

Synergy with other Communities

NSLS has a long history of multi-technique and inter-disciplinary research efforts. This results from unique characteristics of the facility: the broad spectral range spanned by the VUV-IR and X-Ray rings enables a diversity of techniques not found at many other synchrotrons; Friday lunchtime seminars introduce staff and users to other applications of synchrotron science; the intimate but open environment on the floor puts life scientists in close proximity to researchers from other fields; and perpetual understaffing persuades users and staff to share resources to accomplish their goals.

Regardless of exactly how the intra- and inter-disciplinary synergy became a hallmark of NSLS, the user community hopes to continue this tradition and expand it to the concept of a Biology Village at NSLS-II. This mode of operation will both produce new research ideas and ensure optimal use of resources.

For example, in structural biology, the amalgamation of intra-disciplinary techniques such as MX and XAS helps one understand the high-resolution fine structure of metal-binding sites in metalloproteins. SAXS and XAS can combine to determine the molecular structure of biomolecules that will not crystallize. SAXS and nano-tomography can be combined to determine the nanoscale structure of biological complexes and molecular machines. SAXS and x-ray footprinting will combine to study the dynamics of protein folding, ligand-binding, and enzyme catalysis.

For chemical and structural imaging, the suite of imaging tools that will be put in place at NSLS-II will exploit the unprecedented new capabilities that NSLS-II will offer for improved sensitivity, spatial resolution, and imaging speed. By developing these capabilities in an integrated suite, all-important correlative microscopy capabilities and sample preparation facilities will be incorporated, with maximum compatibility in specimen handling and mounting. The environment of this suite will enable cross-fertilization of ideas among these three research communities, enabling new advances. For example, in Alzheimer's disease, the nanoscale structure of tau tangles and amyloid plaques can be visualized in solution with SAXS and in cells or tissue with

nanotomography; the secondary structure of the plaques can be determined with infrared imaging; metal-ion uptake into the amyloid plaques can be probed with the hard XRF microprobe; and the plaques can be visualized in vivo with DEI. These studies can be combined with structural biology methods such as XAS to determine metal-binding in vitro, SAXS and x-ray footprinting to understand the dynamics of metal-binding and plaque formation, and MX to determine the atomic resolution structures of the enzymes involved in the production of the amyloid protein.

Taken together, structural biology and chemical/structural imaging can translate basic science research into medical applications and the discovery of new drugs. On one hand, it can take advantage of the current development of "generic assays" to identify ligands that bind specifically to a biomolecular target. On the other hand, these ligands could then be used as probes in chemical genetic approaches and take advantage of the imaging capabilities. The "Biology Village" would serve as a catalyst to enable investigator-driven research and provide new pathways for discovery. It would also capitalize from two ongoing NIH-efforts – the protein structure initiative and the molecular libraries of chemicals.

In addition to multi-technique applications, the life sciences community has a strong overlap with other research fields. For example, the environmental sciences community has many common interests with biological sciences in topics of universal interest, including environmental toxicology and human health, nutrient cycling and soil pollution, the interaction of nanoparticles and colloids with human cells, radioactive materials, and contaminant transport and diffusion processes. Thus, the community anticipates that the life sciences suite of beamlines will also be of interest to the environmental sciences users, especially in the areas of XAS, STXM, IR imaging, and XRF microprobe. Conversely, the life sciences community also anticipates the need to utilize other specialty beamlines including soft x-ray XAS, quick-XAS, single crystal XAS facilities, and micro-diffraction for cutting edge and specialized research projects.

Finally, it should be noted that Brookhaven National Laboratory is an ideal environment for life sciences research beyond synchrotron techniques. In addition to the current and future synchrotron facilities, there are strong programs in positron emission tomography (PET), magnetic resonance imaging (MRI), Cryo-electron microscopy, and multi-modal optical imaging facilities at BNL.

MACROMOLECULAR CRYSTALLOGRAPHY

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Overall Summary

Macromolecular crystallography has transformed our understanding of biological processes. The routine use of synchrotron radiation for single crystal diffraction studies has revolutionized macromolecular structural biology. With the availability of brighter x-ray sources, the size and complexity of macromolecules that can be studied has increased by an order of magnitude. The expectation that a deeper understanding of biological processes can be achieved once the structures of ever more complicated structures and complexes are known has been the major driving force in the continuing development of synchrotron radiation facilities worldwide. To date, crystallography continues to be the dominant method for determining the three-dimensional structure of increasingly larger and more complex molecular structures. The source of this increasing role of synchrotron radiation is the large x-ray energy band width to help with phasing, the high collimation to resolve diffraction patterns from very large molecules, and the high overall intensity to make the measurements go quickly, even for small, weakly diffracting crystals.

Biomedical research has entered a new era, with an increasing emphasis on understanding the functional and physical connections between macromolecules. Integrative biology is a broad conceptual paradigm for working out how the molecular components of cells and tissues are connected together in biochemical pathways, cellular responses, and functioning organs.

1. Introduction to Scientific Theme

Macromolecular crystallography is the gold standard for structural biology, providing the finest possible detail. The power of the method has driven researchers to attempt increasingly difficult scientific problems, especially structures of large macromolecular assemblies and membrane proteins. Indeed, the size and complexity of macromolecules that can be studied has increased by an order of magnitude in the past 15 years. The size of crystalline specimens also has decreased by this amount during that period. The principal scientific challenge is to continue along this path – larger complexes; smaller specimens.

Large molecular assemblies

Several past successes in molecular structure determination of large complexes and assemblies are indicative of the critical role of high-brilliance, high-flux synchrotron sources in these studies. Early applications were the atomic molecular structure determination of virus particles, consisting of 180 or more proteins with a total mass of 8-9 Mdalton (including the RNA

genome). These studies illustrated the difficulties in working with crystals of large complexes. Weak diffraction, close spacing of the reciprocal lattice, and radiation-sensitive crystals, all conspired to make x-ray data collection only possible at synchrotron radiation sources, Every area of biology has many similarly large macromolecular assemblies that carry out the processes both within and outside the cell, as every process is dependent on the intricate association of these molecules. The challenge for future structural biological studies will be to make these assemblies in large enough amounts to allow their crystallization and to have the synchrotron x-ray sources capable of allowing their structures to be determined even when material is scarce and unstable.

Membrane proteins

Membrane proteins are ubiquitous and essential to all living cells. They are found in eukaryotes and prokaryotes, as well as some viruses, and correspond to a sizable fraction of the entire genome. Membrane proteins are involved in every aspect of cellular function. Our understanding of membrane proteins is far behind that of other proteins. Only a few dozen structures of such molecules are known, a small fraction of one percent of the total. The major limitation to membrane-protein structure determination is still difficulty in production of crystals. However, some of these problems may be overcome through molecular biology; new expression systems, advances in detergent biochemistry, and new crystallization approaches, for example monoclonal Fab-mediated crystallization, increase success. Nonetheless, repeated examples show us, firstly, that the rewards for structure determination of membrane-bound proteins can be great, and secondly that this work depends strongly on frequent access to a bright x-ray source to provide the constant feedback between the synchrotron and the biochemistry lab that is essential.

Structural Genomics

The availability of complete genome sequences for many organisms stimulates the imagination of all biologists, not the least structural biologists. Confronted with the several tens of thousands of genes, nearly all coding for cellular proteins, a structural biologist may want to crystallize them all. That might not be far from the motivation behind the different approaches to this lode of protein-sequence information, each being a facet of Structural Genomics. Certainly, since proteins are central to almost all aspects of biology and disease, Structural Genomics will have an impact on the way biological problems are addressed. A completely different goal might be to obtain a global view of the "protein structure universe" through the identification of the total protein-folding "space"

Two distinct experimental activities are anticipated: crystal screening and definitive structural determination. The current state of the crystal-growing art is such that approximately 20 crystals are screened *per* target protein to identify the truncated and/or mutated form, and the cryo-preservation scheme, that yields the best diffraction. A significant experimental commitment will be required to support screening of small crystals for diffraction properties. Each optimally productive undulator beamline has the capacity to examine approximately 250 to 500 crystals during each 24-hour day when equipped with bar-coded, automated, cryogenic sample changing and crystal centering. NSLS-II undulator beamlines dedicated to structural biology can be used to

handle this load. In this scheme, crystals will be screened for quality; if quality is adequate, sufficient data to solve the structure will be measured and all results recorded in an experiment-tracking database.

Drug Design

One of the most critical challenges faced by pharmaceutical crystallographers, compared to academic researchers, is that the macromolecules studied are very often human proteins, since the aim is to treat human diseases. Human proteins can be very difficult to work with, and the growth of large single crystals can be daunting. Another distinct feature of pharmaceutical crystallography is the time course. Only after the first structure is determined can one begin the cyclic sequence of structures typically used to hone the chemistry of the lead compound, hoping to generate the potency, solubility, and pharmacokinetic properties required of a drug. In this phase of the program, it is essential that crystallographic feedback be rapid and accurate. Synchrotron-based crystallography helps not only with optimization of crystallization methods, but also to solve new structures easily. The synergy between the needs for automation and high throughput within both pharmaceutical research and structural genomics is clear. Each can benefit from the other's automation developments in genomics, crystal formation, and x-ray data collection. The development of convenient access to efficient synchrotron facilities is central to both efforts.

2. The Growth, Expansion, and Transition of NSLS Scientific Programs

Currently the largest user group at NSLS, the macromolecular crystallography community represents 40% of all NSLS users. The scientific achievements of this community are extraordinary, with 55% of its publications in premier journals, and approximately 75% of all NSLS premier publications in the last two years. New York City is the center for two major NIH Structural Genomic initiatives, *Northeast Structural Genomics Consortium* and *New York SGX Research Center for Structural Genomics*, two specialized centers, *New York Consortium on Membrane Protein Structure* and the *Center for High-Throughput Structural Biology,* and one homology center *New Methods for High-Resolution Comparative Modeling*. Home to several universities, the northeast is seeing an expansion of its biomedical resources that will profit from the increased capabilities provided by the new source.

NSLS has been contributing to industrial research over 15 years. It actually pioneered the concept of an MX beamline dedicated to the pharmaceutical sector. In the last couple of years pharmaceutical firms have recorded about 10% of the total productivity, measured as numbers of diffraction images recorded. Plans to better meet the needs of pharmaceutical users at NSLS and NSLS-II are being considered.

Most MX beamlines are being maintained to meet a five-to-seven-year lifetime at NSLS; some are being upgraded to meet the possibility of being moved to 3PW stations at NSLS-II. Funding is being sought by some user groups for upgrades of conventional detectors and possibly the purchase of a state-of-the-art pixel-array, active-pixel detector for one of the undulator

beamlines. The MX community supports the idea that three 3PW stations at the new source should be equipped with instrumentation transitioned from the current NSLS MX beamlines.

3. Proposed Suite of Beamlines

Given the character of the research carried out by the MX community, nine beamlines are proposed for NSLS-II. The beamlines should provide the capabilities for high-throughput crystallography and for the development of complex problems.

Six undulator beamlines, built in low-β straight sections should be state-of-the-art, providing the smallest brightest beam possible with the best possible specimen handling to exploit this beam. They should be dedicated to the most challenging problems, e.g. weakly diffracting crystals, large unit cells, and small specimens, and allow the use of ancillary techniques. It is proposed that these beamlines complement each other in energy range and sample environment. By using canted undulators in one straight, these six undulator beamlines could be served by three straight sections in the storage ring. The experimental station on one of each pair of canted beamlines would probably be smaller and perhaps less versatile.

Three beamlines should be built on 3PWs as conventional MX beamlines. These might be constructed from usable components of the existing ten NSLS MX beamlines. Equipped with conventional MX instrumentation, these beamlines could fulfill the conventional needs of the community. One of the stations should be devoted to high-throughput measurements, dedicated to structural genomic problems and drug design, and this beamline could also fulfill the routine needs of the pharmaceutical sector.

A possible layout that shows two undulator beamlines and one 3PW beamline for MX is shown in Figure 1.

4. Beamline Specifications and R&D Needs

a. Source – The sources required are mentioned above.

b. Optics – A conceptual design of a tunable macromolecular crystallography undulator beamline was described in the NSLS-II CDR. It incorporates a cryogenically cooled double silicon crystal monochromator followed by a K-B focusing mirror system.

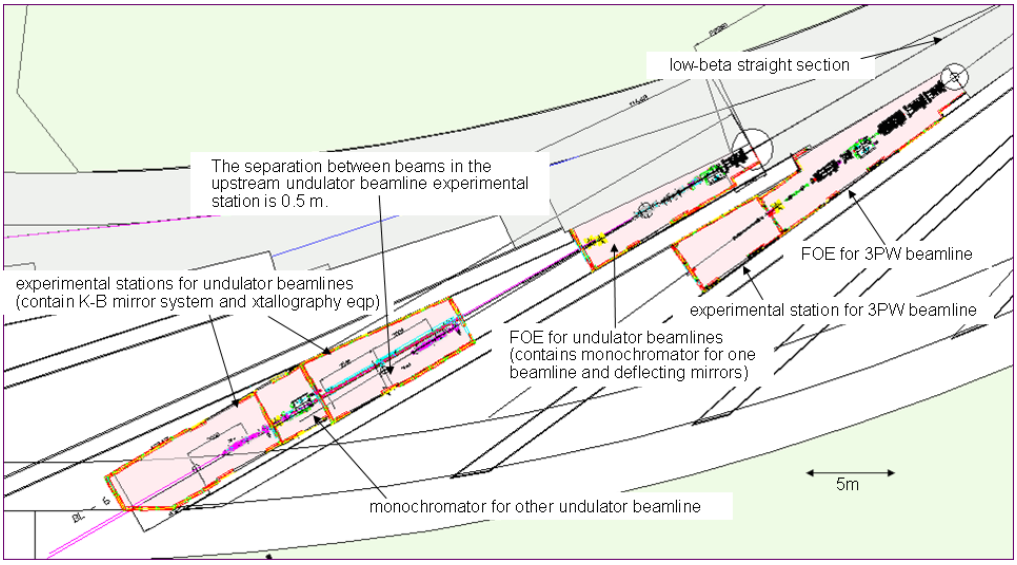

Figure 1. Concept for layout of two undulator-based beamlines (left) and one 3PW beamline (right) in one sector.

c. Experimental apparatus – Already, motorized crystal manipulators are capable of few-micron positioning. The community would benefit from access to an active-pixel detector system that could frame at 100 Hz with about ten million 50-micrometer pixels.

d. R&D Efforts – sub-micron resolution beam position and profile sensing; thermometry and temperature stabilization of the x-ray beam footprint on the first crystal; adaptive compensation of the distorted wavefront using downstream corrective optics; evaluation of diamond as a substitute for silicon; mirror polishing and metrology.

e. Upgrades to beamlines for transit – Detector upgrades, replacement of mirrors in otherwise useful mirror drives.

f. Automation of the optics and sample environment

Acknowledgments: Leemor Joshua Tor, Wayne Hendrickson, Marc Allaire, Lonny Berman, and all who contributed to previous documents.

SMALL/WIDE-ANGLE X-RAY SCATTERING

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X-ray scattering has been an important tool for the life science community to study non-crystalline structures at various levels of complexity, from biomolecules in solution, membrane structures, to tissues such as bones and muscles. The unparalleled source brightness at NSLS-II will undoubtedly enable measurements that are not possible today. At the same time, the growing interest in understanding how biological molecules and molecular assemblies function under physiological conditions has rapidly increased the demand for routine solution scattering measurements. A successful transition to NSLS-II should therefore provide not only new capabilities for brightness-limited measurements, but also higher capacity and better utilization of available beam time for routine solution scattering measurements. Furthermore, it is important to recognize that scattering measurement is only one of the many tools that life science researchers utilize. Flexible access to beam time, such as mail-in service and rapid access, and availability of supporting facilities are therefore very valuable for the life science community.

1. Overview of x-ray scattering in life science research

INCREASING DEMAND FOR BIOMOLECULAR SOLUTION SCATTERING

Small angle solution scattering can detect large conformation changes in biomolecules due to ligand binding or change in chemical environment. Comparison of experimental solution scattering data and that calculated from high-resolution structures provides validation for crystal structures and allow for rigid body modeling to study how subunits interact in larger structures. In addition, the computation methods developed in the past decade now allow structural biologists to obtain *ab initio* low-resolution shape envelope of molecules from solution scattering data. These low-resolution envelopes have been subsequently used to help structural determination by crystallography and cryo-EM. Other applications of solution scattering data and low-resolution shape envelope, such as data analysis combined with NMR data and sequence-based structural prediction under the constraint of the shape envelope, are being actively pursued.

While solution scattering measurements usually focus on the low scattering angles that correspond to length scales relevant to the overall shape of the molecule, recent studies have begun to explore the information embedded in scattering data at wide angles pertinent to secondary and tertiary structures. Such information may be useful to classify protein structure into fold families, and as a sensitive tool to detect small conformation changes, for instance due to ligand binding, that are not visible in small angle scattering.

Solution scattering measurements are relatively simple to perform and its new applications have been attracting more and more structural molecular biologists, a community that makes up the vast majority of the life science users at NSLS today. With the large number of proteins being produced by structural genomics efforts and new methods of making use of solution scattering data being developed, the demand for solution scattering is expected to increase dramatically in the coming years.

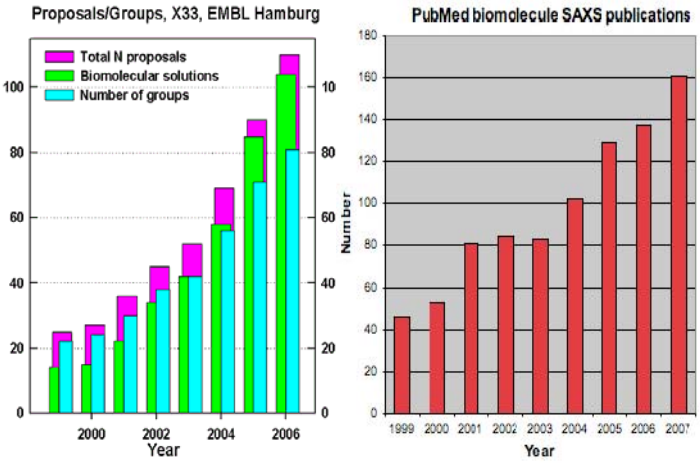
NEW SCIENCE ENABLED BY NSLS-II SOURCE BRIGHTNESS

Many x-ray measurements on biological samples require low x-ray divergence in order to achieve low scattering background. For samples that also require small x-ray spot size on the sample, the measurements become source brightness-limited. There are several areas in life science research that will benefit from the brightness of NSLS-II the most.

Time-resolved scattering from biomolecules in solution. Kinetic studies are essential to understand the functions of biological molecules. Micro-fabricated continuous flow cells have been demonstrated to be able to achieve ~10μs time resolution. However, in solution scattering measurements, the potential of these devices can only be fully realized using beam size of a few microns. For time-scales from milliseconds to sub-second that are relevant to protein enzymatic activities, stopped-flow mixing devices are more practical. An undulator-based SAXS beamline at NSLS-II is expected to deliver up to 10^{15} photons/sec into the beam spot, compared to $<10^{11}$ photons/sec at NSLS today. The duration of a single measurement therefore will decrease from tens of seconds today to milliseconds.

Two-dimensional membrane structures. Structural studies of membrane proteins by crystallography have had very limited success because these proteins are intrinsically not suitable for production of 3-dimensional crystals. Instead, studying membrane proteins in 2-dimensional membranes that resemble their native environment is an attractive alternative. X-ray diffraction from membrane proteins, both in crystalline states and as 2-dimensional solutions, is currently being explored. Successful measurements require small x-ray beam size (microns or less in one direction) because the sample size is usually small and these measurements must be performed in grazing incidence geometry that magnifies the beam spot size on the sample.

Hierarchical structures in biological tissues. Biological tissues often exhibit hierarchical structures, with relevant length scales ranging from angstroms to microns. X-ray scattering using micron-sized beam spots allows for measurements to probe molecular and supramolecular organization underlying structural features observable in optical microscopy. Micro-beam x-ray scattering can also be used as a scanning probe microscope that provides direct structural information complementary to other forms of microscopy, such as IR or x-ray fluorescence, that provide chemical information.



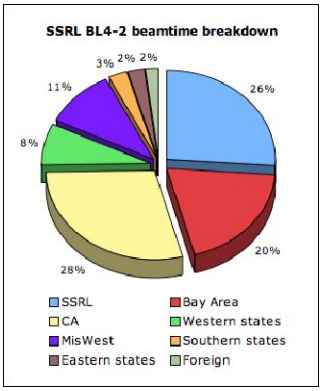
Trend of increasing beamtime requests (statistics from EMBL beamline X33 at HASYLAB , the beamline where a lot of the new solution scattering analysis methods were developed) and number of publication solution scattering in recent years.

2. Growth, Expansion and Transition of NSLS Scientific Programs

The community believes that biomolecular solution scattering should be the focus of growth and expansion in the transition to NSLS-II. The demands for this technique and the number of publications that utilize solution scattering data have been increasing in recent years. Since many of these measurements are not limited by source brightness, a large, experienced, productive user community can, and should, be built up today and be ready to use the new facility by the time NSLS-II starts to operate.

Today, only beamline X21 supports solution scattering measurements at NSLS, utilizing 5-10% of the total beam time. This is in sharp contrast with 80% at beamline 4-2 of SSRL and 35% at the BioCAT beamline 18 ID at the APS, both which are small angle scattering beamlines dedicated to life science research. (An additional 20% of the time at BioCAT is used for closely related areas such as small angle fiber diffraction. BioCAT also has also maintained an oversubscription rate beftween 60% and 100%.). The low usage of solution scattering at NSLS is partially because SAXS is only one of the three experimental programs hosted by X21. This situation is expected to improve when X9 begins to operate as a dedicated SAXS beamline. But the issue of multiple research fields getting their fair share of the same beamline must be addressed. This is particularly important when one is attempting to develop user communities. For instance, it may be desirable to assign a fixed minimum percentage of beam time to each research field and adjust them from time to time based on actual demand in order to ensure that a particular user community retains sufficient access to remain viable.

Growing the solution scattering user community will require a combination of development of high-throughput capability to better utilize the limited beam time available and better staff support to attract new users. Solution scattering measurements are usually straightforward and take relatively short time (less than a minute per sample at the current NSLS). However, analysis of scattering data can be often intimidating for life science users who are new to this technique. Furthermore, in life science research each technique often provides one of the many pieces of information needed to solve the problem. Many users may therefore prefer to have solution scattering as a service to provide the information they need. This is similar to motivation behind the successful mail-in



An example of locality of solution scattering users.

program that currently exists for protein crystallography. Having a program like this certainly will help the growth of the solution scattering community.

The growing need for x-ray scattering techniques for life science research is being recognized world-wide and several SAXS beamlines dedicated to biological research are under construction. The availability of these new beamlines is unlikely to affect the demand for solution scattering capacity here in the US. Statistics show that users tend to use facilities close to their home institution. For instance, ~75% of the users at beamline 4-2 at SSRL are from California. The active structural molecular biology research community in the Northeastern US will provide the user base for solution scattering at NSLS and NSLS-II.

3. Proposed Suite of Beamlines

Given that x-ray scattering in life science research encompasses a large variety of samples and different variations of experimental methods, the community believes that three different x-ray scattering beamlines are required, at the minimum, to meet the anticipated research needs.

3PW beamline dedicated to solution scattering. This beamline is intended to meet the increasing demand for a fully dedicated, high-efficiency solution scattering instrument. A multilayer monochromator should be used to make up for the lower source brightness. Capabilities for high-throughput measurements and simultaneous detection of small- and wide-angle scattering are required. The beamline should also be equipped with non-x-ray-based protein characterization tools such as dynamic light scattering and UV and IR spectroscopy to provide more complete characterization of the protein samples and to ensure that only good samples get measured. The beamline is expected to have slightly better flux performance compared to a BM beamline with similar optics at the current NSLS. There is already insufficient capacity for solution scattering nationally to meet the demand. It may be desirable to build this beamline at NSLS and then move it to NSLS-II.

Undulator beamline for high-brightness measurements. This beamline should be able to perform SAXS measurements using a beam size of a few microns for brightness-limited measurements such as those described in the overview section. It should also be able to provide beam size of a few hundred microns for measurements using stopped-flow mixers and chromatography. Radiation damage is the primary concern in these measurements and absorbed dose can be reduced by spreading absorbed x-ray energy into a larger area. Beamline X9 at NSLS has these capabilities and should be considered as a candidate to be moved to NSLS-II, provided that the monochromator is upgraded to handle the higher heat load expected from an NSLS-II undulator. The micro-focusing optics should also be replaced/supplemented if alternatives become available. This beamline should be equipped with a high-quality, in-line optical microscope to help visualize the exact position of the x-ray beam on the sample. Life-science research alone may not be able to occupy the full capacity of this beamline. Sharing this beamline with the soft condensed matter community is practical (such as is done at ID2 at the ESRF) because of the similar instrumentation needs by the two communities.

Reconfigurable 3PW beamline. Some measurements such as fiber diffraction and model membrane diffraction on bulk samples are best carried out at a beamline that can be easily reconfigured for different measurements (e.g. point detector vs. area detector, use of 4-circle spectrometer vs. long beam path, high flux vs. high energy resolution). Again, the soft condensed matter community is likely to have similar needs and can share this beamline.

4. Transition / Construction Sequence

The key of the transition process is to build up the user community today, particularly for the biomolecular solution scattering program. The operation of the new X9 beamline is a good start for this process. Not only will X9 provide more beam time for user experiments, the undulator source will also allow some brightness-limited measurements to be explored. However, more user time also requires more support by knowledgeable staff. The community believes it is of great importance for X9 to have staff support to help attract inexperienced users. As with protein crystallography, solution scattering users very often need beam time when samples become available and therefore require flexible user access modes such as rapid access and mail-in service. High-throughput instrumentation must be included as an important component of these user access modes to relieve the burden on beamline staff and better utilize available beam time. The devices required by high-brightness measurements, such as flow cells that will be used for time-resolved solution scattering and the fast shutter (sub-millisecond), must also be developed. Software development would need to keep pace with high-throughput instrumentation that is expected to generate a large amount of data. In addition, new data analysis methods and software alternatives would have to be explored to resolve open issues in solution scattering analysis, such as the uniqueness of the structure model, and how to deal with data collected from protein mixtures.

The community believes that a dedicated solution scattering 3PW beamline is needed to serve the needs for solution scattering at NSLS-II. The present X9 beamline is a good candidate to be moved to an undulator beamline at NSLS-II. The life science community needs to work closely with the soft condensed matter community to coordinate the sharing of these beamlines.

5. Facility Infrastructure

In addition to lab automation that carries out high-throughput measurements, the community believes that the infrastructure is needed to provide capabilities to produce proteins on site and instrumentation such as FPLC, DLS, and UV-Vis spectroscopy for protein purification and characterization. The success of solution scattering measurements is intimately linked to the quality of the sample. Although many soluble proteins are quite stable and do not aggregate, it is not rare that additional size-exclusion chromatography just before data collection is required to ensure data quality. In the case of membrane proteins and macromolecular assemblies that are often unstable and would not be usable after shipment, it would be critical to have a protein expression and purification facility near the beamlines. The community also encourages the provision of office space and computation facilities for users so that they can perform preliminary data analysis before they leave the beamline.

Clearly, staff support is crucial to ensure the research productivity of the user community. It is important to realize that staff support is different from simply setting up the experiments. For life science research in particular, even experienced users may require assistance and consultation with trouble-shooting problems with samples (e.g. sample purity, radiation damage) and subtleties in data analysis. Beamline scientists therefore must be knowledgeable of these issues by being actively involved in independent research themselves and playing lead roles in the development of experimental and data analysis methods. Having good research infrastructure provides a better chance for staff scientists to carry out their own research, which ultimately benefits the user community.

X-RAY ABSORPTION SPECTROSCOPY

Sandeep Rekhi & Mark R. Chance

Case Center for Synchrotron Biosciences

1. Introduction to Scientific Theme

The multidisciplinary EXAFS technique is widely used to characterize metal ligand structure information. From the biosciences prospective, EXAFS, which relies on synchrotron radiation as the x-ray source, can be used to determine aspects of metalloprotein active site structures at extremely high accuracy for a range of sample states, including powder, crystalline, and solution. XAS can be used independently or to substantiate the structural information of intricate protein systems as derived from x-ray crystallography or nuclear magnetic resonance spectroscopy. This technique can provide element-specific structural information for reaction intermediates of protein samples that are not amenable to investigate as single crystals or on samples that are difficult to crystallize. Determination of high resolution structure and interaction mechanisms at the metal active site can provide the structural basis for drug design, and Bio-XAS plays a vital role in this field.

The main focus of the present life sciences XAS facility (beamline X3B) at NSLS is to support research that probes the structure and function of proteins and other biomolecules, and to develop innovative physical and biological approaches to solving critical problems in the realm of biomedicine. The main users are interested in an energy range of 5 keV to 14 keV, corresponding to the transition metals of interest in that region. The facility offers various academic and industrial institutions access to the synchrotron light source, assists in the design of experiments, actively participates in solving complex problems in biomedical research, and promotes the publication of this pioneering and vital research in renowned journals.

One of the other major in-house research activities at X3B involves metalloproteomics where transition metals bound to newly isolated proteins are identified by detecting their x-ray fluorescence. The metalloproteomics program is collaborating with the Protein Structure Factory (Germany), New York Structure Genomics Consortium (NYSRGC), and the Northeast Center for Structural Genomics. The latter are two of the four large-scale structure genomics centers in PSI-II. So far, the presence/absence of transition metal content has been successfully characterized in over 1500 proteins from nearly 1000 families. The main objective of this program is to develop a metalloprotein annotation database that includes characterization of at least one protein from each of the 5000 largest protein families.

The Bio-XAS program was moved from X9B to X3B in the summer of 2006. Presently, X3B is oversubscribed, and the facility has been, as a matter of course, providing much higher general user experimental run time than is awarded based on the general user minimums extant at NSLS. Thus, we have recently increased the time allocated by the GU program to 50% and we intend to increase this to 75% later in 2008.

The Bio-XAS program at NSLS has been highly productive in both service projects and in-house research. In 2006-2007, more than 40 publications were produced from the X9B/X3B beamline and ~30% of these were published in premier scientific journals.

Most X3B users conduct their experiments on biological samples with very low concentrations of metals (in the micro-molar range). For dilute proteins, EXAFS analysis limits are ultimately dictated by the intensity of x-rays delivered to the sample. The transfer of the Bio-XAS program to NSLS-II can take advantage of the two-fold increase in flux anticipated at NSLS-II as compared to NSLS. Furthermore, NSLS-II will offer a smaller and more stable beam at the samples. These paramount features will allow the acquisition of better quality data and higher sample throughput. Besides running conventional routine XAS on biological samples, NSLS-II will open various possibilities to conduct new research in conjunction with other facilities such as microprobe and diffraction.

2. The Growth, Expansion, and Transition of NSLS Scientific Programs

The X9B (now X3B) beamline went through many major technical improvements over the past ten years to suit life science user demands. Some of the upgrades during the last five years include attaining more flux on the sample, installing a non-ribbed second crystal in the sagittally focused monochromator, modifying the motor control of the second crystal stage, improving the signal detection system, and full automation of the data acquisition system. The X3B beamline is presently equipped with an advanced beam-focusing system collecting 4 or more mr of horizontal divergence, solid-state detection, and fully automated data acquisition system. Integrated with the XAS setup, the new metalloproteomics arrangement constitutes an additional fully automated stage for metal screening that can accommodate up to 11 plates with 220 sample wells at a given time. The total time to acquire a data set on 220 protein samples is ~9 hours. The high precision table that synchronizes the movement of the sample cryostat, detector, ionization chambers and alignment laser has been recently fabricated and installed. This new feature at X3B helps in precise and repeatable swapping of EXAFS and metalloproteomics instruments.

To attract new users and to match X3B with other top-rank XAS facilities, further improvements of the facility are planned. These will extend the energy range to higher energies (such that Mo can be examined), but will not encompass energies outside of the range of 5-22 keV. Some of the foremost upgrades anticipated in the one to two years are a technically advanced monochromator and a state-of-the-art 31-element detector with digital electronics. The technically older double parallelogram linkage design of the present monochromator is a major source of vibration and oscillation, thus degrading its overall performance. To achieve a broader energy range, the swapping of silicon crystals is required, involving significant downtime and potential damage to delicate components. During the next two years, the Bio-XAS facility has proposed to upgrade the present monochromator to one that incorporates all motion controls on a single shaft, upgrading and coupling of the in-vacuum motors for better scanning operations and tunability, and an easier swapping of crystals (with the capability of cryogenic cooling). The life sciences XAS community can utilize the advantages of the new monochromator, including broader coverage of energy (5.0 keV – 22 keV), improved beam stability due to reduced vibrations of scanning

components and a homogenously dispersed beam on the sample. The use of a Si-220 crystal can provide better energy resolution along with a factor of two times more flux using an improved sagittal crystal with larger horizontal beam acceptance. The improved scanning components in the new monochromator will also provide faster and more precise energy scanning and calibration capabilities vis-à-vis the existing monochromator. The upgraded monochromator with a cryogenically cooled first crystal can be easily utilized at the anticipated XAS beamline of NSLS-II. The new system will be helpful in measuring low concentration samples (with larger total active area of detector), increasing the data quality (with faster data acquisition), allowing high-throughput studies (with availability and selection of more regions of interest), and avoiding problems with detector saturation.

The proposed upgrades, along with an increase in the available general user time to 75%, will help in further growth of existing programs as well as promoting initiation of new experiments. With the new instrumentation, even higher throughput will be achieved and the metalloproteomics program will be expanded. The "mail-in" and "drive-by" programs will also be positively impacted by the upgrades. Based on the merit of the proposed experiment, the Bio-XAS facility will also consider granting ~5% of its GU time for bio-environmental sciences projects.

3. Proposed Suite of Beamlines

Considering international trends in Bio-XAS, with the requirements for stable beam and modest flux-density as optimal for Bio-XAS, the life sciences community proposes that a Bio-XAS facility be developed at a 3-pole wiggler source at NSLS-II. Figure 1 outlines some of the major Bio-XAS beamlines in the world today. The current X3B, and X3B projected, is shown in the figure. A beamline delivering in excess of 10^{12} photons/sec with a broad spectrum of radiation (bending magnet or wiggler) would be a suitable facility. One of the ultimate limiting factors is detection and sample damage; thus flux-density is not the most important variable for the experiment.

This facility might be achieved at NSLS-II through a transfer of the program with selected equipment from the X3B beamline and program with all major instruments transferred from the present X3B facility. Future upgrades of the instruments planned at the present Bio-XAS facility should take into account all specifications and requirements at NSLS-II such as space considerations (front-end-enclosure, hutch dimensions, beamline space).

Facility Beamline	Flux (Photons/sec) Energy range	Beam Size (hor. x vert., mm)	Detection system	Conditions
Canadian Light Source BioXAS -1 Wiggler	9×10^{12}	0.69 x 0.13	100 pixel monolithic Ge x-ray array detector	Photon energy = 10 keV, 500 mA,
NSLS X3B (Present)	5.0×10^{11} 5 - 13.6 keV (Si-111)	0.4 x 0.2 focused	13 element Ge-detector	Photon energy = 10 keV, 200 mA
NSLS X3B (Upgraded)	**10^{12} 5.5 keV – 22 keV (Si-111 and Si 220)**	0.3 x 0.1 focused	**31 element Ge-detector**	**Photon energy = 10 keV, 200 mA**
SSRL Station 9-3	~ 2×10^{12} 5 – 30 keV (Si-220)	0.4 x 3.0	30 element Ge solid state array	Photon energy = 9 keV 100 mA
APS 12-BM/BESSRC	8×10^{11} 2.4-22 keV (Si-111)	1 x 1 focused	13 element Ge detector	Photon energy = 10 keV
XOR/PNC-20-BM-B	1×10^{11} @ 10 keV 2.7 – 32.5 keV (Si-111)	1 x 0.05 focused	13 element Ge detector	Photon energy = 16.3 keV,
Spring 8 BL01B1	10^{9} to 10^{11} 3.8 ~ 113 keV (Si -111, Si-311, Si-511)	0.150 x 0.010	19-element Ge solid-state detector	Critical energy 28.9 keV , 0.1 % BW
NSLS-2 3 pole wiggler	3×10^{12}			

Figure.1. Features of leading XAS beamlines around the world.

Also, the community expects that advances at detection, either in commercial instruments or those developed at BNL, will be one of the important factors governing the ultimate performance. To meet the scientific needs, the general user time should be increased to 80% with 20% time for developmental activities.

For cutting-edge experiments and those with different energy range or scanning speed requirements, the life science community would be highly interested in obtaining general user time at the facility XAS beamline (for time-resolved measurements) and at other specialty XAS beamlines for techniques such as quick EXAFS and soft-energy XAS. In the last ten years, Bio-XAS has been a very powerful tool to determine local structure on single crystals in synergy with crystallography. The biosciences group would also like to initiate single crystal Bio-XAS research capabilities at NSLS-II.

Taking into consideration the expected user demand at the primary Bio-XAS facility, the life sciences community proposes that a secondary 3-pole wiggler XAS beamline be shared with the overlapping field of earth and environmental sciences. Environmental science shares a similar set of instruments and experimental protocols with biological science in terms of XAS studies on samples. The Bio-XAS community is anticipated to request 50% of the GU time on this beamline. The detailed plans and funding for such a beamline is undetermined at this moment and will require further discussion among both communities. The commonality between two fields can direct users to investigate issues of universal interest, such as environment and human health, nutrient cycling and soil pollution, interaction of nanoparticles and colloids with human cells, radioactive materials, and contaminant transport and diffusion processes.

4. Beamline Specifications and R&D Needs

The life sciences community foresees one full XAS beamline dedicated to biological samples. Given the projected high demand for access to beamlines and user census, the life sciences community is amenable to participating in an additional beamline shared with the earth and environmental science community. Both of the beamlines should be developed at 3-pole wiggler sources. The life sciences community also anticipates limited participation at the facility XAS and specialty beamlines including soft x-ray XAS, quick XAS, and single crystal XAS facilities for cutting edge and specialized research projects. Depending upon the users' interests, the life science community also envisions the utilization of two or more techniques in conjunction that can be within life sciences disciplines or interdisciplinary scientific communities, for example XRD, microprobe, and imaging coupled with XAS.

As the present Bio-XAS beamline is expected to be transferred to a 3-pole wiggler source at NSLS-II, all the present instrumentation at X3B should be upgraded as necessary during the intervening time to ensure the availability of a state-of-the-art facility for relocation. Besides the upgrades currently planned for the next five years (see section 2), additional upgrades at the Bio-XAS facility may include the introduction of a semi-automatic sample-to-beam centering system, a cryo-sample changer for multiple samples, further upgrades of the detection system either by

adding additional elements to the 31-element detector or adopting a completely new system. The monochromator should be upgraded to match the technology at that time.

5. Facility Infrastructure at NSLS-II

In addition to the usual demands for instrumentation, offices, and lab space, the Bio-XAS facility at NSLS-II envisions the facility to be equipped with cryogen lines near the hutch so that the cryogens are easily accessible for biological samples. The Bio-XAS hutch should contain auto-cryogen filling modules for automatic filling of detectors with liquid nitrogen. The life sciences facility at NSLS-II would also require a machine shop and electronics lab to design and construct beamline-related instruments. Staging areas are needed for transfer and storage of beamline equipment and devices for different experiments. A sample storage area will be required especially to store consignments of bio-samples related to the "Mail-in" program. The hutch should be equipped with auto gas handling units so that gases such as helium, dry nitrogen, argon and air are easily accessible. The biosciences XAS community envisions a facility with a uniform control system interface that offers similar motor control and data acquisition software throughout all XAS facilities at NSLS-II. This will not only facilitate troubleshooting of software-related problems but also grant better networking within the XAS community. The capability for automated data back up at a centralized facility would also be of great interest.

6. Synergy with other Communities

Intra- and inter-disciplinary synergy is very important to produce new research ideas as well as to ensure maximal utilization of resources. The amalgamation of intra-disciplinary techniques such as three-dimensional protein crystallography (PX) and absorption spectroscopy (XAS) is imperative to acquire and understand the high-resolution fine structure of metal-binding sites in complex proteins and has been actively considered by the life science community worldwide. Bio-XAS also has synergy with imaging and microprobe life sciences techniques. On the other hand, the life science and environmental science communities share overlapping research interests and can be synergetic in terms of sharing beamlines, instruments and some sample preparation areas. Since the interests of both disciplines overlay in one portion of a Venn diagram, some part of the research infrastructure can be shared by both communities. The Bio-XAS community supports the idea of a "Biology Village'" where the proximity of synergistic techniques will create an environment not only conducive to utilization of multiple techniques for life sciences but also that promotes the free exchange of scientific ideas within the community.

X-RAY FOOTPRINTING

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1. Introduction to Scientific Theme

Hydroxyl radicals cleave the phosphodiester backbone of nucleic acids and covalently modify amino acid side chains in proteins. In sections where a molecule is folded, however, these sites are inaccessible to solvent, and therefore protected from modifications. X-ray footprinting employs the very intense and ionizing white x-ray beam to generate hydroxyl radicals in microseconds-milliseconds, a timescale appropriate to probing macromolecule dynamics and minimizing sample perturbation. For nucleic acids, one then analyzes the pattern of fragments after x-ray exposure by gel electrophoresis; the protected sections that are not cleaved yield a "footprint". For proteins, the exposed samples are digested with proteases and analyzed by LC- and tandem-mass spectrometry to determine the extent and sites of modification. The data provide detailed structural information (at the single-nucleotide and single side-chain level) that is used to map tertiary structure as well as regions of macromolecular interaction, which can subsequently be used as constraints for molecular modeling to generate high-resolution structures. Time-resolved footprinting can be employed to reveal mechanisms of tertiary contact formation during biomolecular functions such as folding, ligand binding and macromolecular assembly. This method is unique for gaining insight into dynamic processes involving large RNA-protein and protein-protein assemblies at higher resolution on biologically relevant timescales and under physiological conditions. These measurements are achieved with a flow device equipped with a fast mixer to allow triggering of a dynamic event, followed by exposure. The applications of time-resolved footprinting measurements are currently limited by beam flux.

The X28C bending magnet beamline at NSLS, operated by the Center for Synchrotron Biosciences (CSB), is currently the only facility in the world fully dedicated to x-ray footprinting. Due to growing demand for the technique, other synchrotron facilities (ESRF, LNLS) have recently developed beamline facilities that support x-ray footprinting. Beamline X28C at NSLS supports both national and international users through collaborative and general user research projects as well as core research projects for both scientific and technical development. This facility has a long track record of high-quality research, with a significant number of premier publications each year. The overall performance of this facility and the future project plans clearly indicate the necessity and importance of establishing an x-ray footprinting beamline within the life science sector of NSLS-II.

2. Scientific Challenges and Transition of NSLS Scientific Programs

For the past several years, x-ray footprinting has been successfully applied to a variety of biological systems. With the growing interest in studying large biomolecular assemblies and *in vivo* systems it is necessary to obtain a higher flux density beam on the sample to reduce the quenching

effect of hydroxyl radical scavengers and achieve significantly shorter exposure times. Shorter exposure time is the key factor for hydroxyl radical footprinting to obtain high quality data. In addition, the added benefit of the use of a microfabricated ultra-fast mixer (section 3.3.2) will lower the overall instrumentation dead time to hundreds of microseconds for time-resolved studies. In the following section, examples of ongoing collaborative research programs, their current limitations and the need for a new beamline at NSLS-II are highlighted. These collaborative research projects are driving several developments in core technologies as described in section 3.

2.1 Macromolecular Interaction *In Vivo* and in Sub-cellular Compartments

The development of new tools for *in vivo* probing of both nucleic acid and protein molecular interactions are currently being undertaken through a joint research program involving Case Western Reserve University (CWRU) and Johns Hopkins University using x-ray mediated hydroxyl radical footprinting. Currently planned projects expected to benefit greatly from transition to NSLS-II include probing of the molecular mechanism of ribosome assembly inside living cells in real time and investigating the structural changes in the vertebrate visual photoreceptor rhodopsin upon photo-activation inside retinal rod cells. These living cell systems will contain significant amounts of hydroxyl radical scavengers and thus require exposure times of several hundreds of milliseconds at the X28C beamline. Such long exposure times can produce severe perturbations in cells and in the target macromolecule, resulting in poor data quality. Short exposure times are essential to control the radiation-induced alternations that might perturb the cellular machinery under investigation. A transient sub-millisecond or single digit millisecond pulse of highly intense x-rays from the NSLS-II damping wiggler (DW) source will provide the least perturbation of the macromolecular system of any *in vivo* technique so far developed.

2.2 Membrane Protein Dynamics

G-protein coupled receptors (GPCRs) are responsible for the regulation of a myriad of processes in the human body including regulation of heart rate, blood pressure, and glucose metabolism as well as the senses of sight and taste. Rhodopsin, the visual GPCR in the rod cells of the vertebrate retina, is involved in light recognition and activation of the intracellular G-protein transducin. The importance of the study of rhodopsin activation arises from the fact that it is widely thought, due to the high level of sequence conservation that the mechanism by which Rhodopsin is activated by photons as well as the mechanism by which it activates transducin, is shared by other GPCRs and the G-proteins pairs. This project is a collaborative effort with the department of pharmacology at CWRU. One of our plans is to use x-ray footprinting to better understand the detailed mechanism of rhodopsin activation, in particular the detailed conformational changes transmitted across the membrane to the G-protein binding site. Preliminary studies on this system yielded no modification in the membrane protein in detergent, however using a focused beam (increasing the flux density) has recently enabled visualization of modification across the transmembrane region in the purified form. Our main goal is to visualize the conformation dynamics of rhodopsin in its natural environment, which is inside the rod outer segment (section 2.1). At present, this study is not possible even with the focused beam in our current beamline configuration. A higher flux density beam, anticipated to be available at NSLS-II, is required. Along

with development of an ultra-fast mixer (section 3.3.2), this will allow expansion of these and similar experiments to time-resolved efforts.

2.3 Studies of Macromolecular Assemblies

Determination of tertiary contacts under various physiological conditions is important in determining the structure and function of macromolecular assemblies. Crystallization of large complexes is generally difficult and NMR studies become limited due to high molecular weight. CryoEM and SAXS can provide low-resolution global structural information. For these systems, x-ray footprinting is highly advantageous, requiring only micro or sub-micromolar concentrations of sample and maintaining the macromolecule in close to physiological conditions. Current research projects (ongoing/proposed) include structural studies of activation of the Arp2/3 complex (>300kD), interaction between F-actin and myosin (>1000kDa), ClpAP protease assembly (>1300kDa) and Ribosomal assemblies (>1000kDa) in collaboration with AECOM, UCLA, MIT and UCSC, respectively. Macromolecular assemblies, by virtue of the large number of amino acid residues, are themselves strong hydroxyl radical scavengers. In addition, they often require millimolar concentrations of cofactors like NTP/NDP (nucleotide 5'-tri/di phosphate) for their optimum functions, which significantly decrease the concentration of hydroxyl radicals during x-ray exposure. For these systems, an increase in flux density is critical to allow short enough exposure times to obtain first-order dose rates and a high signal-to-noise ratio for overall high-quality data.

3. Proposed New Beamline at NSLS-II

3.1 Source Requirements

X-ray footprinting applications require a stable, broad energy range, high flux source. A 3.5 m canted section of an NSLS-II Damping Wiggler (DW) is an ideal source for this purpose. The DW source produces the highest total flux of all NSLS-II sources in the energy range of interest (5-20 keV), enabling x-ray footprinting experiments to reach much shorter exposure times and investigate previously unapproachable systems.

3.2 Beamline Configuration

Initial designs include a vertically collimating mirror inside the ring at the front end to reduce the head load and filter high energies from the canted DW source as well as reduce the vertical divergence of the beam. The front end is expected to have standard components including a white beam slit to accept up to 1.0 mrad horizontal by 0.15 mrad vertical based on the NSLS-II specifications. The front optical enclosure starts from the shield wall and ends before the experimental hutch. The FOE would house gate valves, bellows, graphite pre-filters as required to protect the Be window for vacuum isolation, water-cooled Al attenuators and a horizontal focusing mirror, the design of which would be based on NSLS-II development plans and specifications for canted DW sources. FOE components would be designed and installed to accommodate proximate optical elements for the second canted beamline.

The experimental hutch is expected to house several interchangeable sample exposure set-ups, a beam monitoring device and adjustable beam slits. Each of the interchangeable sample exposure components would be designed to minimize the time and effort required to set up or reconfigure an experiment. Exposure equipment expected to transition to NSLS-II include a temperature-controlled multiple sample holder unit for sample exposure under steady-state conditions and a flow set-up for time-resolved studies. It is expected that the development of an ultra-fast mixer will have yielded an upgrade to the flow system by the start of NSLS-II. Beam monitoring would be carried out with a visualization system, which provides a field of view large enough to study the beam size, beam profile, and the beam position stability of a focused beam in the end station. Tentative location of the end station is ~35m from the source.

3.3 End-station Development and Upgrades

Several upgrades in the end-station configuration are essential for prompt data collection maintaining the condition required for handling biological macromolecules in solution for both *in vivo* and in *vitro* x-ray footprinting experiments.

3.3.1 Automated Sample Handling and Exposure

The construction of an automated sample exposure and handling mechanism is essential to minimize experimental time in the beamline. Acquiring data or sample exposure in the presence of the focused beam requires precise determination of the size of the beam and the alignment so that the sample is exposed homogeneously as well as exposed within the maximum or peak beam flux density. Redesigning of the beamline alignment apparatus would be necessary for the new canted DW beamline at NSLS-II to accomplish this. Development of this equipment is expected to occur prior to NSLS-II commissioning.

3.3.2 Development of an Ultra-fast Mixer

A very short exposure time is achievable by the use of the high-flux NSLS-II canted DW source, requiring the development of an ultra-fast mixing device to allow the sample to mix and then expose on the hundreds of microseconds time scale. The community hopes to develop or purchase (if available) a device tailored for x-ray footprinting in which rapid mixing is achieved by creating nonlaminar flows before the collision of two solutions. Several such rapid mixing techniques have already been pioneered for different spectroscopic and scattering studies and have been widely adopted. Construction of such a mixing device requires micro fabrication using appropriate substances that will have high x-ray permeability, high thermal conductivity, and low retention characteristics for biological molecules.

3.4 Automation in Data Analysis

Footprinting experimental methods have been continuously improved and now represent a routine set of protocols in the CSB laboratories that have been widely disseminated throughout the world and employed by many user groups. Currently, the process of protein footprinting MS data analysis remains a tedious, manual procedure of peptide identification, extraction, and quantization. This barrier represents a bottleneck that limits the future growth of the method. Our current goal is to speed this process of data analysis for CSB collaborator projects and subsequently

expand the capabilities of the software to permit the analysis of a wide variety of mass spectrometry data. This conversion from manual to automated data analysis is only possible due to the availability and application of high-resolution mass spectrometry instrumentation now available to CSB collaborators and staff through the Case Center for Proteomics at Case Western Reserve University.

4. Footprinting Facility Infrastructure

Experiments for the proposed beamline require laboratory space for spectroscopic equipment, an electrophoresis facility, wet chemistry and sample preparation areas, a cell culture facility, a sample storage freezer, and a cold room. The spectroscopy area should have adequate space to hold two mass spectrometers coupled to liquid chromatography systems, UV-visible and fluorescence spectrometers. Some sensitive samples for *in vitro* and *in vivo* studies require close proximity of wet-lab spaces to the beamline. In addition, an instrumentation laboratory for ongoing beamline upgrades and development will be necessary. Sufficient office space also will be needed to house the 6 FTEs required per beamline.

5. Synergy with Other Communities

The local changes in solvent accessibility deduced from the profiles of hydroxyl radical reactivity can be integrated effectively with static atomic structures obtained from x-ray crystallography as well as with global reporters of biomolecular structure such as small-angle scattering, CryoEM, and other biophysical techniques to provide a complete picture of macromolecular transitions. Several such examples are highlighted in the following paragraphs.

5.1 Denaturation Assays and Footprinting

Techniques such as urea- or ion-dependent denaturation assays followed by UV-vis, fluorescence, CD, or gel electrophoresis can be combined with footprinting to generate site-specific unfolding isotherms. These can lead to an understanding of stability changes at the molecular level. This approach allowed a quantitative determination of differences in free energy for the formation of specific tertiary contacts of RNA under different solution conditions. A similar approach was used to evaluate the linkage of ion-induced folding of the RNA.

5.2 SAXS and Footprinting

The combination of local structural information provided by synchrotron hydroxyl radical footprinting with global measurement techniques such as SAXS has already proven valuable for understanding processes of macromolecular folding. The hierarchy of ribozyme folding was observed on the millisecond timescale using footprinting, and time-resolved small-angle x-ray scattering studies have shown that an overall collapse of the RNA to a compact structure precedes formation of the native tertiary contacts. In combination, the techniques describe a relationship between tertiary contact formation and compaction, indicating that folding of this large RNA is a subtly tuned process that ultimately results in biological function. In another system, analyses of SAXS data were consistent with a three-stage hypothesis of Ca^{2+} activation of gelsolin provided by footprinting data and provided details on the predominant structures of the intermediate Ca^{2+}

bound states supported by this hypothesis. Together, the synchrotron footprinting and small-angle x-ray scattering studies highlight the value of obtaining both global and local measurements.

5.3 X-ray Crystallography and Footprinting

Many footprinting experiments focus on protein/protein interactions in large protein assemblies. Crystallographic data has often enriched footprinting studies by providing details at atomic resolution for individual components of such complexes. For example, AVP protease was modeled bound with its activation factors, cleaved C-terminal peptide and viral DNA. Also, monomeric Mg^{2+}-G-actin structure was modeled from its footprinting data. Footprinting data has also been used in combination with docking to predict and model actin/cofilin interaction. Thus, in several ways footprinting and x-ray crystallography are complementary techniques.

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UV CIRCULAR DICHROISM & RELATED SPECTROSCOPIES

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1. Scientific Theme

Synchrotron radiation (SR) is the source-of-choice for all spectroscopic measurements requiring intense, broad-band radiation in the vacuum ultraviolet (VUV) – photon energies greater than about 6 eV or wavelengths less than about 190 nm. Circular dichroism (CD), the difference between the absorption left and right circularly polarized light, is especially useful in characterizing naturally chiral molecules ranging in size from four atoms up to large proteins and other complex biological macromolecules. MCD, which is CD induced by an applied magnetic field, requires similar instrumentation, but is complementary in providing information on achiral (symmetric) systems. In addition, a beamline capable of measuring CD can also measure the absolute absorption of a sample and luminescence generated by absorbed light. Thus, a single beamline can address the needs of three distinct communities.

STRUCTURAL BIOLOGY, BIOPHYSICS AND BIOMEDICAL RESEARCH

Proteins consist of amino acids linked by peptide bonds that have conformation-sensitive CD spectra for wavelengths extending from about 240 nm to at least 160 nm. CD spectra can quantify the secondary structure of proteins and peptides *i.e.*, the fraction of the peptide bonds in α-helix, β-beta sheet, and other conformations. Compared to conventional source instruments, SR increases the high quality spectral range by about 40% and has been demonstrated to increase significantly the accuracy of determining secondary structure. Synchrotron-radiation-determined CD (SRCD) has also been demonstrated to improve the quality of the VUV CD spectra of nucleic acids (DNA and RNA), polysaccharides, and carbohydrates. The latter are particularly important because they usually begin exhibiting CD at ever lower wavelengths, which are even less accessible to conventional CD instruments, and because or their importance in current research on biofuels.

The structural biology/biophysics community has been the largest user of CD spectroscopy at NSLS. Although originally demonstrated in the US, SRCD has developed extensively in Europe, where the SRCD beamlines at Daresbury and Aarhus, which focus on biological applications, have operated at saturation for the past decade. Other SRCD beamlines are operating at Berlin, Hiroshima, and Beijing and are planned for Diamond, Soleil, NSRRC (Taiwan), and Melbourne. Interest in SRCD studies of bio-macromolecules is increasing in the US as a result of publications

from outside the country and periodic workshops and short courses conducted since 2005 at BNL. Currently, there is no operational stopped flow on any SRCD worldwide, so the addition of this capability would be both unique and a major attraction to new users.

MOLECULAR SPECTROSCOPY

This research emphasis is on small molecules, particularly in the vapor-phase or matrix isolation, and involves both MCD, which is observable in both chiral and achiral molecules, and natural CD, which is restricted to chiral molecules. The experimental setup differs from that for biological applications only in the area of the sample chamber, with both the up– and down-stream components being identical. In the case of MCD, the "sample chamber" must include an appropriate magnet. The potential user community is smaller, but we are fortunate in being joined by Professor Patricia Snyder, who has been involved in the CD/MCD beamline at Aladdin. Few SRCD studies of biomolecules have extended to wavelengths less than 160 nm due the absorption of water, not the performance of the spectrometer. For vapor-phase and matrix-isolated CD/MCD experiments, sample absorption is not such an issue, and operation to ~120 nm is anticipated. This limit results from the polarization modulator, and can possibly be extended by the use of a LiF modulator if one can be built that performs satisfactorily (see below).

MATERIALS CHARACTERIZATION

Scientists and engineers with diverse interests sometimes need to characterize the optical properties (typically the unpolarized absorption spectrum or luminescence excitation/emission/polarization spectra) or photochemical properties of some compound or material in the VUV. Off-the-shelf spectrophotometers are not available for wavelengths less than 170 nm, and even in those situations where a conventional source might do the job, the cost of assembling a system from scratch is prohibitive for a typical "one-off" experiment. Existing VUV beamlines at NSLS automatically record unpolarized absorption spectra in addition to CD, and numerous users have used this capability to record absorption spectra or test photochemical responses. For example, a firm that makes polarization modulation equipment used in semi-conductor foundries will in the near future use beamline U11 to determine the absorption spectra of doped LiF crystals in the VUV. When no measurement of polarized light is required, absorption measurements can be extended to shorter wavelengths (~ 80 nm) if a UHV sample housing is employed. Solid anisotropic samples can also be studied using linear dichroism or reflectance. The electronics to perform LD experiments have been implemented at U11 as a matter of quality assurance. Additional optics and detectors for recording luminescence are also available. Engagement with the emerging nano-materials community at BNL is anticipated.

2. NSLS Scientific Program: Growth, Expansion and Transition

At present, two beamlines at NSLS are devoted to the VUV spectroscopies described above – U9B and U11. We propose only one VUV/polarization beamline at NSLS-II, and propose to concentrate all upgrades and relevant capabilities on U11, which has superior VUV penetration. Thus, all upgrades and development work would be focused on U11.

NEW USER COMMUNITIES

Much of the research involving **nanomaterials** involves characterizing materials using various forms of spectroscopy. The VUV beamline fills what would otherwise be a gap in the spectral coverage provided by NSLS. As noted above, users interested in characterizing materials already make some use of existing VUV beamlines. Engaging the nanomaterials community *via* seminars or other presentations is likely to increase the participation of such users.

3. Proposed Beamlines for NSLS-II

A single VUV beamline based on the existing U11 is proposed. No secondary beamline is anticipated. No de nova VUV beamlines are anticipated for NSLS-II.

BEAMLINE SPECIFICATIONS AND R&D NEEDS

We expect that the NSLS-II source will be a bending magnet beamline with high horizontal acceptance, i.e. a modified infrared configuration. The beamline optics would be a two-element monochromator consisting of a plane mirror plus focusing grating. The endstation would have interchangeable sample compartments with kinematic positioning. Since a superconduction magnet may be used for certain experiments, potential adverse effects on other beamlines/equipment will be considered. In the short term, we plan to continue the development of a stopped-flow system and investigate the possibility of multiple wavelength detection.

UPGRADES FROM NSLS

The VUV beamline needs the largest possible horizontal acceptance. As of January 2008, this appears to require an "IR" bending magnet configuration. The present design indicates that a plane mirror will be located just outside the ring to deflect the beam upward. While the VUV beamline, unlike an x-ray beamline, can tolerate large angle deflections, the number should be limited to the greatest extent possible. The upward deflection therefore suggests an optical configuration such as used successively at Daresbury and Århus in which the concave grating also deflects the beam by 90°. This locates the end station on a platform above other beamlines, while retaining a "two optical elements" configuration. Such configurations should be considered.

FOURIER TRANSFORM INFRARED MICROSCOPY

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1. Introduction to Scientific Theme

Fourier transform infrared microscopy has become a valuable technique for examining the chemical makeup of biological cells and tissues on a microscopic scale without the need for stains or labels. Synchrotron infrared (IR) light is an ideal source for the technique due to the combination of its high brightness (i.e. flux density) and broadband nature. Through a 10-micron pinhole, the brightness of a synchrotron source is 100-1000 times higher than a conventional thermal (globar) source. The high brightness leads directly to improved spatial resolution (to the diffraction limit of 2-10 μm in the mid-infrared region) and data quality (i.e. signal-to-noise).

With the high spatial resolution of the synchrotron, individual cells within a tissue can be probed with sub-cellular resolution, imaging the molecular chemistry of disease. For example, the structure of misfolded protein aggregates has been identified in the brain tissue of Alzheimer's disease patients [1, 2] and infectious prion proteins have been characterized in scrapie [3-6]. Variations in bone composition have been observed as a function of age [7], in osteoporosis [8, 9], osteopetrosis [10], and osteoarthritis [11]. In heart disease, altered lipid and collagen content and structure in the myocardium have been seen [12], which were partially normalized by losartan treatment [13]. The high spatial resolution of a synchrotron IR source permits the subcellular chemical mapping of single living cells for the first time. Sample heating has been shown to be negligible, permitting analysis of single cells for time scales from hours to days [14, 15]. Individual mouse hybridoma B cells have been examined during necrosis and the end phases of mitosis [16], and also during the process of apoptosis [17]. Spectral differences have been seen between normal and cancerous oral epithelial cells [18, 19], healthy and nutrient-repleted *Micrasterias hardyi* algal cells [20], and HepG2 cells exposed to low doses of 2,3,7,8-tetrachlorodibenzo-p-dioxin [21]. Variations in DNA/RNA content and packing have also been demonstrated during the cell cycle of human lung epithelial cells [22].

2. The Growth, Expansion, and Transition of NSLS Scientific Programs

Infrared microscopes are typically built to perform either Fourier transform infrared micro-spectroscopy (FTIRM) or imaging (FTIRI). In an FTIRM experiment, an aperture confines the beam to the sample's area of interest and a second aperture is used after the sample to define the region being sensed by the single-pixel IR detector. For such a "confocal" microscope, the spatial resolution is $\sim \lambda/2$, which is 1 – 10 μm in the mid-infrared region [23]. The high brightness of the synchrotron source and the confocal arrangement of the microscope provide a spatial resolution unsurpassed by any conventional laboratory instrument because of the high brightness of a synchrotron IR source.

However, data collection is time-consuming because they utilize a single-element IR detector. Raster-scanned images of a single biological cell can take more than an hour to collect, and subcellular imaging of significant regions of tissue can take several days.

In an FTIRI experiment, no physical apertures are used to limit the illumination area of the IR beam. Instead, an array of IR detector elements is used to collect the projected image of the unmasked IR beam on the sample. The focal plane array (FPA) systems dramatically improve the rate at which IR images can be collected, but the spatial resolution is not as good as a confocal FTIRM microscope because an FTIRI instrument cannot operate in a confocal arrangement. However, will the excellent signal-to-noise provided by the high brightness of NSLS (and added stability of NSLS-II), image deconvolution methods are possible, which could improve the spatial resolution of the technique beyond the diffraction limit, i.e. to < 1 μm.

NSLS currently has five synchrotron infrared microscopes, making it (by far) the largest synchrotron infrared imaging effort worldwide. Three microscopes are operated as Facility Beamlines by NSLS (U10A, U10B, U4IR) and two microscopes are operated by PRTs (U2A, COMPRES; U2B, Case Center for Synchrotron Biosciences). Four of these microscopes operate in the confocal (FTIRM) configuration. The fifth microscope, and the newest addition to the NSLS family, is the Bruker Hyperion 3000 located at beamline U4IR. This instrument, which was funded in 2007 by an NIH Shared Instrumentation Grant, is equipped with at 128x128 pixel FPA.

Three of the infrared microscopes at NSLS are primarily used by life sciences researchers (U2B, U4IR, U10B) and all are fully or oversubscribed. In fact, beamline U10B is among the top three most requested beamlines at NSLS and is 2x oversubscribed even though it offers 75% of its beam time to general users.

To date, an FTIRI microscope has not been permanently installed on a synchrotron source, largely due to optical constraints of previous instruments. However, efforts are currently underway at NSLS and elsewhere to couple an FTIRI microscope to a synchrotron infrared beamline. Specifically, we are currently designing and constructing the coupling optics for synchrotron operation of the Bruker Hyperion 3000 at U4IR. This path is clearly the direction for many synchrotron infrared imaging experiments in the future; these instruments will dramatically improve data collection rates and enable dynamic measurements of living cells in culture at a spatial resolution unsurpassed by laboratory instruments.

3. Proposed Suite of Beamlines

We propose that the initial complement of beamlines at NSLS-II match the existing NSLS VUV-IR beamlines at time of transition. For that, we anticipate the following:

- 2 mid-IR microprobe beamlines for biological, environmental, materials science, and space science (U2B, U10A, U10B)
- 1 mid-IR beamline for biological and chemical imaging using FPA detectors (U4IR)

- 1 far-IR beamline for magneto spectroscopy (U4IR)
- 1 mid- and far-IR beamline for materials science, including time-resolved spectroscopy (U12IR)
- 1 mid- and far-IR beamline for studies of materials at extreme temperatures and pressures (U2A)

We expect that all infrared beamlines would extract dipole radiation from bending magnet ports at NSLS-II. We would like the front-end design to follow design concepts similar to the existing IR extraction from the VUV-IR ring. The balance of the beamline components would have a more robust design to exploit the lower noise environment of the NSLS-II facility.

For most synchrotron storage ring sources, the intrinsic brightness is determined entirely by the circulating beam current. However, designing an optical extraction configuration that preserves the brightness, while meeting mechanical and accelerator design constraints, can be quite difficult. Typically, the large angular collection necessary to achieve an acceptable performance involves non-standard construction geometries for the dipole chambers. We are planning two designs for extracting infrared from NSLS-II dipoles (**Figure 1**). One design is based on a large gap (60 mm) dipole for collecting approximately 50 milliradians of horizontal source arc and from 23 to 40 milliradians vertically from the front half of a dipole bend. This extraction provides far-infrared performance comparable to the existing NSLS VUV-IR far-infrared ports. The 2nd design is similar except that the dipole magnet will have a conventional gap, reducing the available vertical extraction by a factor of 2. In both designs, the second dipole in a DBA cell would be used to avoid the ID beamline front ends.

The collection efficiency is determined by the natural opening angle for synchrotron radiation in the long wavelength limit. For a wavelength, λ, and bend radius, ρ, the half-angle is defined as:

$$\theta_\lambda = \left(\frac{3}{4\pi} \frac{\lambda}{\rho} \right)^{1/3}$$

Because of the large bending radius (~ 25 meters), the infrared from NSLS-II dipoles is emitted into angles 2.35 times smaller than for the existing VUV-IR ring (~1.9 meters). Thus, the extraction performance for NSLS-II with 38 milliradians would be identical to a 90-milliradian port on the VUV-IR ring. A study of the NSLS-II dipole design indicates that horizontal extraction of 50 milliradians is available. However, the small dipole gap and vertical chamber dimension (about 25 mm) will limit the vertical collection to approximately 16 milliradians. While this is adequate for mid-infrared spectroscopy for chemical imaging, it limits the performance for far-infrared spectroscopy. Therefore, a second dipole magnet design is planned that would be used where far-infrared ports are required. This dipole will have a ~ 60 mm gap and will accept a dipole chamber with approximately 32 milliradians of vertical collection.

Because mid-IR microprobes only require about 15 milliradians of vertical and horizontal collection, at least 2, and possibly 3 such beamlines could share a single, conventional-type dipole chamber. The mid-infrared imaging will need the entire 50 milliradians of horizontal collection, so each beamline would require its own dipole (conventional-type). The far-infrared beamlines would also each require a full dipole extraction as well as the modified, large gap dipole and chamber to collect the ~ 32 milliradians of vertical aperture. In terms of the overall NSLS-II storage ring, a design where 5 conventional dipoles have mid-IR extraction capability, plus 5 large-gap dipoles allow for far-infrared extraction, will give us the capacity to meet the anticipated needs at the time of the transition from NSLS to NSLS-II along with a ~ 40% growth capacity to meet future beamline needs.

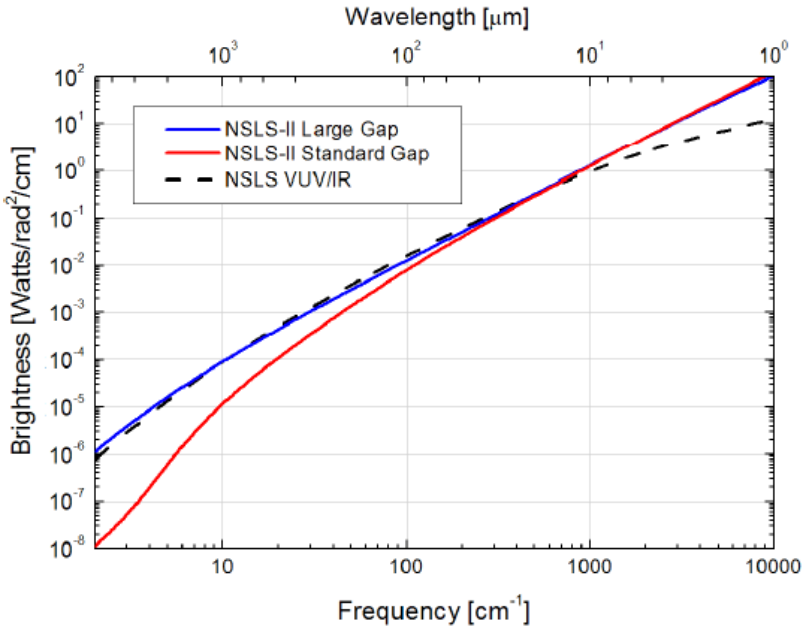

Figure 2. *NSLS-II infrared brightness for standard gap (red) and large gap (blue) dipoles compared to the current brightness of NSLS.*

4. Beamline Specifications and R&D Needs

IR imaging with an FPA: For FPA imaging, a large horizontal swath of beam is required to produce an extended source with significant depth. Although segmented mirrors have been shown to allow for such a large angular collection to be manipulated and matched to a flat focal plane array detector, this approach may also introduce undesirable multiple-beam interference effects. Therefore, we will also investigate designs for extraction mirrors having a varying curvature along meridian. Although the mirror surface may be complex, computer-assisted, diamond-turned optics are expected to meet performance requirements. We also envision other anamorphic optics to smoothly re-shape beam profile to optimally match the square format of FPA detectors.

Top-up mode: At NSLS, even though IR beamlines do not have photon shutters, users typically pause their data collection during injection due to noisy data. Since NSLS-II will operate in top-up mode, an acceptable method for operation FTIR instruments must be determined. Specifically, most infrared beamlines utilize commercial Fourier-transform infrared (FTIR) spectrometer systems. These systems and their commercial software would need to be interfaced

with NSLS-II injection signals to avoid collecting data during a top-off cycle. Converting all the systems to an EPICS-type interface will be considered.

5. Recommended Transition/Construction Sequence

For the life sciences, it is expected that endstations at beamlines U10A, U10B, and U4IR will remain state-of-the-art while currently operating at NSLS, and will be transitioned to NSLS-II once NSLS has produced its last photons. Since infrared beamlines are rather straightforward (compared to x-ray beamlines), and infrared endstations operate with commercial software, we anticipate that the transition will take < 3 months to complete so that user downtime will be negligible.

6. Facility Infrastructure at NSLS-II

Hutch enclosures: One major oversight at the current NSLS was the construction of IR beamlines without a climate-controlled hutch enclosure. Infrared spectra are extremely sensitive to interferences from water vapor and CO_2 absorption; thus, humidity control is critical for successful experiments. (In fact, many users prefer to come to Long Island in January to collect their data; even though the beaches are much nicer in the summer, the water vapor in their spectra in August can be debilitating.) For NSLS-II, we would like to implement complete enclosures for beamline endstations and instruments. The primary purpose of these hutch enclosures is to provide a stable climate (temperature and especially humidity), control acoustic noise from neighboring beamline systems, and provide for a dark environment when performing optical alignment. These enclosures would be designed to be occupied at all times, i.e., they would play no role in any radiation protection system. However, they could also serve for access control to hazardous experimental conditions such as laser light, high magnetic fields, and biological toxins.

Additional infrastructure requirements for the biological IR community include: a wet-lab with cell culture facilities nearby; a microscope lab with access to light microscopes and microtomes; and a computer lab for real-time data analysis and processing.

7. Synergy with other Communities

Infrared microscopy is a technique used by a wide range of user communities, including life sciences, environmental sciences, soft matter, chemical sciences, materials science, and condensed matter physics. While current NSLS IR beamlines serve this wide range of users, endstation equipment is often specialized for certain types of experiments in narrower fields of research. Thus, we would like to see the IR beamline at NSLS divided up into research "villages," where FTIRM and FTIRI microscopes are available in the Life Sciences Village for those users. In this vision, other IR microscopes would be strategically positioned around the ring to accommodate other researchers such as those in condensed matter physics.

For infrared microscopy, the life sciences community overlaps strongly with the environmental sciences community, where endstation requirements, sample preparation, and applications are all similar. Thus, we anticipate that the infrared beamlines built for life sciences will have the structure and capacity to accommodate the environmental sciences community, too. Moreover, the interactions between these two groups will provide an excellent environment for discussion and collaboration, opening the door to new areas of research at the interface of these fields (e.g. environmental toxicology).

Lastly, infrared microscope users at NSLS have a great advantage over many other synchrotrons because they also have access to a number of other microscopy techniques at the same facility. It is very common for IR users to also run their samples on the STXM microscope (X1A), the hard x-ray microprobe (X26A, X27A), the microdiffraction beamline (X13B), and/or the DEI beamline (X15A). No other synchrotron in the US has this suite of capabilities, and only a few can claim this achievement worldwide. The life (and environmental) sciences users at NSLS have become accustomed to this wide range of capabilities, and we would like to see it continue and expand at NSLS-II.

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X-RAY FLUORESCENCE MICROSCOPY

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1. Introduction to Scientific Theme

Although XRF microprobes have existed in many synchrotron facilities, their recent introduction to the life science community has already generated tremendous interest. XRF microprobe (and possibly nano-SIMS) is the only technique that provides sufficiently high elemental sensitivity at micron or better resolution required for studies at cellular and tissue level. In addition, chemical speciation can be revealed on the same specimen using micro-XANES analysis. This technical development coincides with a growing interest in life science to study trace metals. In biology, one-third of all known proteins contain a metal cofactor, and they regulate many essential cellular functions (e.g. differentiation, division, transcription, apoptosis). Not surprisingly, dysregulation of metals has been linked to a number of diseases (e.g. Wilson's, Menkes, Alzheimer's, Parkinson's, Lou Gehrig's) and tumor growth (angiogenesis). Thus, there is an urgent need to study metalloproteins at the cellular and tissue level in homeostatic and pathogenic states. Also, many exogenous, or "bad" metals (e.g. Cr, As, Cd, Pb) are classified as carcinogens, but their toxicology and cell biology are not well understood. In medicine, metal-containing drugs have been used for treating cancer, arthritis, leukemia, diabetes, and malaria, even though their cellular distribution and transformation are not well understood. Similar studies may also be very important in order for the emerging field of nanomedicine to gain acceptance, where nanocomposites (e.g. TiO_2) are employed for diagnostic or therapeutic purposes. And in microbes and plant science, samples are often heterogeneous from micro- to nano-meter scale, and local chemical speciation is the determining factor for many reactions (e.g. transport and redox reaction of metals and environmental contaminants mediated by microbes).

Since the performance of scanning microprobe depends directly on the source brightness, all research areas outlined above can hugely benefit from the use of high brightness synchrotron sources such as NSLS-II. Currently, there are 6-7 XRF microprobes at the Advanced Photon Source, serving the geology, environmental, and life science users with micron and submicron beams, which together provide beam time equivalent to three full-time microprobe beamlines. However, they are currently all oversubscribed by 2-4 times.

2. The Growth, Expansion, and Transition of NSLS Scientific Programs

The two XRF microprobes at NSLS, beamlines X26A and X27A, can provide a 5-15 micron beam and have been used primarily for earth and environmental science. The X13B diffraction microprobe has demonstrated higher resolution, but is used mainly for materials science applications. Thus, the life science user base for the NSLS-II XRF microprobes would need to be built and expanded in the next few years, perhaps most suitably with the growing effort at beamline X27A. In parallel, technical development of detectors, scanning stages, microprobe control, cryogenic sample environment, and analysis software would need to be undertaken. However, given the highly subscribed programs at X26A and X27A, other beamlines at NSLS may need to be used for the community and technical development. For example, strong consideration should be given to developing beamline X5 as a biological/environmental microprobe beamline that can be directly moved to NSLS-II.

Advanced detector development is probably the most important component for future XRF microprobes. With the increased brightness of NSLS-II, the count rate of XRF detectors needs to be increased by 2-3 orders of magnitude. Also, current single-element, energy-dispersive detectors can only accept 3-5% of the 4π fluorescence, leading to an unacceptable 95% signal loss. Thus, detectors that can handle > 10^7 count/sec and covers > 30% of 4π will be needed. Fortunately, the NSLSNSLS Detector Group (P. Siddons *et. al*.) has recently demonstrated a 96-element detector array, and is in an excellent position to develop advanced XRF detectors required for NSLS-II. In addition, the segmented detector developed at BNL and successfully used at X1A and the APS can detect differential phase contrast (DPC) signal and provide valuable structural context for biological specimens.

3. Proposed Suite of Beamlines

Life science microscopy research at NSLS-II will involve spectromicroscopy, nanotomography, and trace-element mapping. For trace-element mapping, we propose the following suite of hard x-ray beamlines for NSLS-II:

It is envisioned that two undulator beamlines will be needed, one with medium resolution (50-1000 nm) and one with high resolution (< 50 nm), for biomedical studies. We believe they should be complemented by two or three microprobes built on a wiggler source with micron resolution, shared by earth, environmental, and life science users. The need for an intermediate energy microprobe (1-5 keV) should also be considered.

1. A pair of dedicated hard x-ray microprobes for the life sciences community would provide highly focused flux at medium (50-1000 nm) and high (< 50 nm) resolution. The medium-resolution microprobe would be the main tool for tissue and cellular analysis, while the high-resolution microprobe would be for subcellular analysis at the single organelle level. We would like the beamlines to be equipped with both multilayer and crystal monochromator for high flux or high-energy resolution (XANES) studies. The beamline would be designed to deliver very

stable beam over many hours and during energy scans. K-B mirror optics or Fresnel zone plates would be used for microfocusing, and > 10^{11} photon/sec could be expected at the focus spot. With such an intense focus, cryogenic sample environment would need to be implemented at both beamlines. Techniques that could be supported include μ-XRF, μ-XAFS, and XRF tomography. The endstations would be designed for vibration and acoustic isolation, and temperature and humidity stability. This would be a world-class facility for functional studies of metals in biology and medicine.

2. We also envision a pair of focused x-ray probes utilizing canted undulator sources in a single sector developed and managed by an interdisciplinary collaboration between members of the earth, environmental, and biological communities. The proposed sector would best address the interdisciplinary research needs of these communities for next-generation studies, while offering a new technical paradigm that will vastly improve scientific productivity. To achieve the required breadth of capabilities, we propose placing a Kirkpatrick-Baez-mirror-based microprobe on one of the canted undulators with an energy range between 4-25 keV, spatial resolution adjustable from 1000 nm down to 100 nm, and instrumentation for XRF, XAFS, XRD and fluorescence microtomography. On the second canted undulator, we would place a zone-plate-based microprobe with an energy range between 2-15 keV, a target spatial resolution of 20 nm, and instrumentation for XRF and XANES. Sample mounting and registry systems would be incorporated to facilitate sample transfers between the two microprobes.

3. We propose to transition beamline X27A to a 3-pole wiggler (3PW) for a microprobe with micron resolution. This beamline would accommodate new biomedical users and current earth and environmental users at X26A and X27A with higher resolution, higher flux, and higher throughput. Beam-sharing would be implemented at a wiggler port, and the individual beamlines would be equipped with high heat load multilayer and crystal monochromator for high flux or high-energy resolution (XANES) studies. K-B mirror optics would be used to focus ~ 10^{12} photon/sec to a micron spot. Techniques that would be supported include μ-XRF, μ-XAFS, μ-XRD, and XRF tomography. For radiation-sensitive specimens, sample-cooling capability would be required. With continuous upgrade, it is possible that the X26A and X27A endstation could be transitioned to NSLS-II.

4. The need for an intermediate-energy microprobe (1-5 keV), for the studies of Na–Ti (K shell) and Ni-Cs (L shell), should be evaluated.

4. Recommended Transition/Construction Sequence

Beamline X27A could be transitioned to a 3-pole wiggler source at NSLS-II. In addition, the community believes that a canted pair of XRF undulator beamlines are needed to serve the life and environmental sciences communities. One beamline should be based on KB focusing optics (high flux, broad spectral range, 1 μm spatial resolution) and the second beamline should be based on zone plate focusing optics for high spatial resolution (50 nm or better).

5. Facility Infrastructure at NSLS-II

For biological XRF microprobe experiments, a Bio Safety Level 2 (BSL-2) laboratory would be needed with the following equipment:

- biological workbench
- incubator, centrifuge, refrigerator/freezer
- optical fluorescence microscope, confocal microscope
- plunge-freezer, freeze-dryer, high-pressure freezer, cryo microtome, freeze-substitution station
- possibly an electron microscope

Technical expertise in operation of these instruments for users would be essential. Additional office space nearby for staffs, postdocs, students, and technicians also would be required.

6. Synergy with other Communities

As already demonstrated at NSLS, XRF microprobe and IR microscope can provide complementary information, which can greatly enhance the end result. Other important components include optical fluorescence microscopes and confocal microscopes with well-developed labeling and staining techniques. Correlative microscopy will be more and more important in the future.

X-RAY SPECTROMICROSCOPY AND NANOTOMOGRAPHY

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1. Introduction to Scientific Theme

We are fortunate to live in an era of rapid developments in cell imaging, with exciting developments in light microscopy, and in tomography of frozen hydrated samples as thick as ~0.5 μm with energy-filtered cryo electron microscopes. However, there are certain regimes of cell imaging that only x-ray microscopes can address. X-ray microscopes can deliver 30 nm resolution images of overall structure (without labels), and they can image whole, unsectioned eukaryotic cells with a thickness of 5-50 μm depending on x-ray energy and contrast mode. By acquiring image sequences at closely spaced energies across an x-ray absorption edge, they can obtain information not just on the presence of a certain element but its chemical binding states; for example, at the carbon edge, one can distinguish between many different organic functional groups when present at about 1% local concentration or higher. By acquiring a tilt series of images or lensless diffraction patterns, they can be used to obtain quantitative, 3-D views of whole cells. The potential exists for extending these capabilities to 10 nm resolution or better in the future. These capabilities are complementary to information delivered by light and electron microscopes, and also to trace element maps via x-ray fluorescence microscopy and organic functional group maps via infrared microspectroscopy. For these reasons, soft x-ray spectromicroscopy and nanotomography play a crucial role in life science research at NSLS in the short term, and at NSLS-II in the longer term.

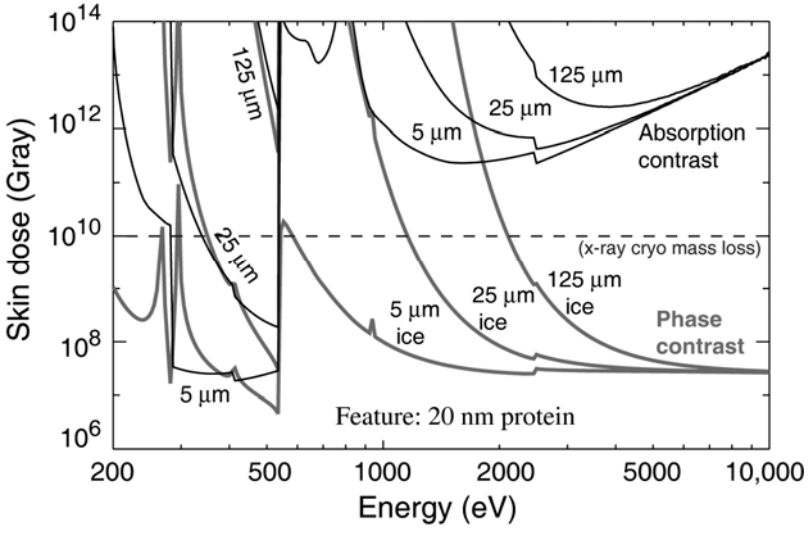

Fig. 1: Theoretical minimum radiation dose required for x-ray imaging of protein in ice.

These capabilities are provided by soft x-ray microscopes operating over the energy range from 280 eV up to intermediate energies of

about 3 keV (Fig. 1). At the lower end of this range lies the carbon *K* edge, where near-edge absorption resonances in the 285-295 eV range can be used to map the distribution of organic functional groups. While additional chemical imaging opportunities exist at the nitrogen (400 eV) and oxygen (540 eV) edges, photon energies just below the oxygen edge provide maximum contrast for transmission imaging of smaller whole cells. At energies above about 1500 eV, phase contrast comes into play, offering capabilities for imaging thicker cells with greater x-ray penetration.

Three microscope types are considered here:

1. In full-field transmission x-ray microscopes (TXM), a Fresnel zone plate is used to produce a magnified image on a 2-D detector. Because all of the pixels of an image are acquired in parallel, these microscopes deliver fast imaging times even with bending magnet sources; at present synchrotrons, this makes TXM the system of choice for acquiring the hundreds of images needed for nanotomography. However, because the 5-15% efficient zone plate is located after the specimen, the radiation dose on the sample is increased 7-20× beyond the other two microscope types described below. Most present examples of these microscopes (including at the Advanced Light Source) use large diameter zone plates as condensers/monochromators with poor spectroscopic capabilities and a lower numerical aperture than the objective lens. However, new commercial instruments such as those used at Stanford, and a cryo TXM at BESSY II, are pointing the way to the future by using conventional x-ray monochromators followed by capillary condenser lenses to deliver higher condenser numerical aperture and true spectroscopy capabilities.

2. In scanning transmission x-ray microscopes (STXM), a beam illuminates a Fresnel zone plate to produce a point focus through which the specimen is scanned. To achieve the finest possible focus, a coherent beam is required so these microscopes are best operated from high-brightness beamlines. The requirement for a coherent beam means that the monochromator need only work with low étendue, making it easy to obtain high-energy resolution for spectromicroscopy. By placing the 5-15% efficient zone plate upstream of the specimen, and a high-efficiency detector downstream, radiation dose to the specimen is minimized. The modest brightness of the present NSLS means that the two STXMs on undulator beamline X1A require minutes to deliver a single image; however, with NSLS-II, the image time might be measured in seconds so that one can consider using STXM for lower-dose tomography.

3. In x-ray diffraction microscopy (XDM) or coherent x-ray diffraction imaging (CXDI), a coherent beam is used to illuminate the specimen (or, in ptychography, a series of portions of the specimen) and the non-crystal diffraction pattern is collected on an area detector downstream. This approach removes any limits of lens resolution, but at a cost of losing the phasing of scattered radiation that lenses provide. Instead, numerical algorithms are used to phase the diffracted data, based either on prior knowledge of the specimen (such as that it is localized to a small area in an otherwise empty field) or on knowledge of the shaping of the coherent illumination beam. While this approach is still in the early phases of its development, it has already been used to image whole cells at 30 nm resolution. XDM/CXDI

offers an important additional advantage over lens-based x-ray microscopes: it is free of any lens-based depth of focus limitations through the reconstruction of a true exit wave which can be numerically backpropagated through the specimen (one can also obtain pure projection images by reconstruction of Ewald sphere tilt series data resampled to lie only on the transverse plane in Fourier space).

All of these microscope types deliver 2-D transmission images. However, their real utility lies in adding a third dimension:

1. In spectromicroscopy, a series of images is taken at closely spaced photon energies around an absorption edge to yield spectrum-per-pixel data. This data can be used for mapping not just the concentration of a particular element, but its various electronic binding states, which reflect its various chemical bindings.

2. In nanotomography, a series of images is taken over a range of closely spaced tilt angles. These images are then combined together to yield a 3-D reconstruction of the object. While a natural consequence of high-resolution x-ray microscopy of thick specimens is that a single image contains considerable overlap of various structures, tomographic reconstruction removes this overlap by delivering slice-by-slice images that can be quantitatively analyzed.

Finally, both TXM and STXM are dependent on optics for delivering high-resolution images. For the 280-3500 eV soft x-ray range considered here, Fresnel zone plates are the optic of choice. The resolution of these diffractive optics is determined by the width of their finest, outermost zones, and zone widths of 25-30 nm are achieved in long working distance, high-efficiency optics today. Zone widths of 15-20 nm have been demonstrated (albeit with poor working distance and efficiency), but there is a consensus in the field that a sustained, long-term effort at optics development might yield 10 nm resolution zone plates in the future.

Like electron microscopy and macromolecular crystallography, x-ray microscopy involves high radiation doses to the specimen so radiation damage is a concern. As in the former two techniques, the solution to this concern is to use cryo techniques to look at frozen hydrated specimens.

2. The Growth, Expansion, and Transition of NSLS Scientific Programs

Scanning transmission x-ray microscopy (STXM) was pioneered by a group at Stony Brook University, and the program has evolved from using a bending magnet source on the UV ring, starting in 1982, to using the X1 undulator on the x-ray ring, beginning in 1989. This group has led the development of spectromicroscopy for nanoscale imaging of chemical speciation, and developed the first cryo STXM worldwide (receiving an R&D 100 award in 1999; however, this microscope proved unwieldy to operate and it was removed in 2002 while planning for a second-generation instrument begun). The X1 undulator now supports two room-temperature STXMS on beamlines X1A1 and X1A2, with one microscope being used almost exclusively for carbon XANES spectromicroscopy while the other is used primarily for oxygen edge imaging in absorption and phase contrast. While the emphasis of the Stony Brook group has shifted in large part to developing

cryo x-ray diffraction microscopy at the ALS, the STXM user program at X1A is fully subscribed and includes some biological research even though only room-temperature operation is available.

Cryo methods are essential for studying life science specimens. In electron microscopy, it has taken decades of development to arrive at good solutions for both microscope technology and sample preparation. Cryo x-ray microscopy can certainly benefit from these developments (and from the expertise of Brookhaven experts in cryo electron microscopy), yet there are important differences, including the fact that sample or optic scanning is required for minimal-dose lens-based x-ray imaging, and that x-rays are able to study samples measuring tens rather than tenths of micrometers thick. During the transition period from now until NSLS-II operation, we believe that the following developmental efforts should be undertaken:

- A second-generation cryo STXM should be developed and installed at beamline X1A2. This will provide an opportunity to develop the scanning cryo microscope instrumentation that will be needed for both soft x-ray microscopes and hard x-ray nanoprobes at NSLS-II. Along with the cryo TXM proposed below, it will provide an opportunity to develop the expertise in preparing cryo specimens optimized for x-ray microscopy (cryo sample preparation equipment outlined in the Facility Infrastructure section of this document would be shifted to the NSLS-II cryo prep lab when NSLS-II begins operation). Most importantly, it would provide unique-in-the-world scientific opportunities to use soft x-ray spectromicroscopy for studies of biochemical organization in whole and cryo-sectioned cells.

- A cryo TXM should be installed on an x-ray bending magnet beamline for phase contrast imaging at energies in the 2-5 keV range. While TXM involves higher radiation dose to the specimen than STXM or XDM/CXDI, it also can deliver fast imaging times with bending magnet sources. This would provide the best way for NSLS researchers to establish a program in nanotomography of frozen hydrated cells. By operating with phase contrast at 2-5 keV, this system would nicely complement the ALS's program in 0.5-2 keV cryo TXM tomography by offering the ability to work with much thicker cells such as oocytes in developmental biology research. In addition, the system should use a high-resolution x-ray monochromator and capillary condenser optic so that it can be used for higher energy spectromicroscopy.

Both of these instruments will allow pioneering new science to be carried out at NSLS, and they can both be transitioned to NSLS II when it begins operation.

3. Proposed Suite of Beamlines

Life science microscopy research at NSLS-II is expected to involve spectromicroscopy, nanotomography, and trace-element mapping. Trace-element mapping in x-ray microprobes is discussed in a previous chapter; for spectromicroscopy and nanotomography, we propose the following suite of beamlines for NSLS-II:

1. A scanning soft x-ray microscopy beamline using an EPU45 undulator, and a beam switching mirror to allow alternating operation of two cryo-capable microscopes over the energy range 280-3500 eV. One of the two microscopes would be used primarily for life and environmental science research. Because of the unprecedented brightness of NSLS-II, this STXM should offer single-image times of seconds. This will make it possible to realize the dream of using STXM for both spectromicroscopy *and* reduced-dose nanotomography. For phase contrast tomography at higher energies, we note that the Stony Brook group has recently pioneered quantitative phase contrast imaging in STXM using specialized detectors and reconstruction algorithms.

2. An x-ray diffraction microscopy/coherent diffraction imaging beamline using a U19 undulator, with two end stations. One endstation would be intended for biological imaging at energies as low as 2.5 keV. This endstation would share with other NSLS-II microscopy beamlines the need for a cryo specimen transfer system.

3. A scanning soft x-ray microscopy beamline using a bending magnet source at NSLS-II, generated by moving NSLS beamline X1A to NSLS-II. While this microscope beamline would not offer world-leading capabilities, the existing X1A microscopes remain scientifically productive even though higher performance microscopes are available today at the ALS and elsewhere. This beamline would also add two essential functions to a suite of imaging beamlines: it would allow users to acquire the reference spectra of standards that are essential for spectromicroscopy data interpretation, and it would provide a platform for the evaluation of various sample preparation protocols, both without taking away time from the higher performance beamline.

4. A cryo TXM on a 3-pole wiggler beamline, generated by moving the proposed new cryo TXM at NSLS to NSLS-II. This microscope would offer rapid nanotomography capabilities over a broad energy range (3-10 keV) for ongoing scientific programs, and would also provide a platform for evaluating the quality of cryo specimen preparation protocols on thicker samples than could be studied on the undulator beamlines.

This set of beamlines would provide a complete range of life science imaging capabilities at NSLS-II, allowing x-ray microscopy research to be dictated not by what subset of instruments are available but by what is needed to address the scientific problem at hand.

4. Beamline Specifications and R&D Needs

We believe that several technical advances must be made prior to NSLS-II operations:

Optics: The NSLS-II R&D plan is centered on developing optics towards a goal of 1 nm focusing of ~10 keV x-rays, which will be a significant achievement. However, the optics being developed for this goal (multilayer Laue lenses, and kinoform refractive lenses) are not well suited to full-field imaging, nor to easy energy tuning or operation at the lower energies that are also required for life science x-ray microscopy research. NSLS-II R&D activities should be complemented with the development of higher-resolution zone plate optics.

Cryo specimen handling: For x-ray nanoprobe studies, flash-freezing can lock diffusible ions into place. For microscopy and tomography studies, specimens must be maintained at cryogenic temperatures so as to maximize the amount of structural information that can be obtained before radiation damage effects are observed. Cryo specimen handling approaches that are compatible with both tomography and with scanning microscopy should be developed,. The high throughput of NSLS-II microscopes means that robotic cryo specimen exchange is a must.

Detectors: In x-ray nanoprobes, NSLS-II will make new demands on fluorescence detectors in terms of collection efficiency (so as to minimize radiation damage by collecting more of the signal) and count rate (because of the increased x-ray flux). In addition, phase contrast detectors are required to put elemental concentrations into their ultrastructural context and to provide accurate concentration information.

Data analysis: because NSLS-II will enable synchrotron-based microscopes and microprobes to deliver more information on biological specimens that are heterogeneous on nanometer length scales, improved data analysis techniques must be developed to deal with data of rich complexity.

5. Facility Infrastructure at NSLS-II

Besides the beamlines themselves, we believe there is a serious need for sample preparation laboratories and correlative microscopy capabilities at NSLS-II. These labs are described in the section on Facility Infrastructure at NSLS-II earlier in this document. They should include a Biosafety Level 2 (BSL-2) wet lab with cell culture facilities, centrifuge, refrigerator, freezer, and sterile laminar flow hood. There should also be inverted and confocal light microscopes with fluorescence and phase contrast capabilities. Because cryo-vitrification methods will grow in importance as the spatial resolution of x-ray nanoprobes and microscopes is improved, a cryo-prep lab with plunge and high-pressure freezers, freeze dryers and freeze substitution unit, cryo-ultramicrotome, cryo-light microscope, and liquid nitrogen storage would be required. A computer lab would also be needed for data analysis and processing beyond what is done in real time at individual beamlines.

Correlative microscopy is taking on a growing role in modern research, by providing cross-validation of results and by providing a means to consider biological questions over multiple length scales, from organ to tissue to cell to molecule. Involvement of Brookhaven's electron microscopy community in both biology and in materials science, and MRI and PET imaging programs, can lead to a powerful set of correlative modalities.

6. Synergy with other Communities

There is a strong commonality of needs between life science research, and organic environmental science as well as soft matter research. In environmental science, leading questions include the role that bacterial exudates play in sequestering and changing the oxidation state of metal and radionuclide contaminants, thereby changing their bioavailability. These questions

involve as much microbiology as inorganic chemistry, and sample preparations and cryo sample requirements much like in life science research. Similar statements can be made about soil science, where studies include the uptake and processing of carbon in the soil, including in organic coatings on clay particles. Soft matter research questions include phase segregation in polymers, and their changes upon hydration; once again, cryo spectromicroscopy and nanotomography can provide important new research capabilities with essentially the same instrumentation requirements as in life science research.

MEDICAL IMAGING & RADIATION THERAPY

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1. Introduction to Scientific Theme

Medical imaging and radiation therapy have a long tradition at the NSLS superconducting wiggler beamline X17B. In fact, three of the five major medical research applications of synchrotron light were initiated and developed at NSLS. These include the microbeam radiation therapy (MRT) and the photon activation therapy (PAT) programs, which were initiated at X17B1, and the diffraction enhanced imaging (DEI) program, which was initiated at X26 and was transferred to X15A. Furthermore, although the two other programs were both initiated at Stanford Synchrotron Radiation Laboratory, their full implementation occurred at the NSLS X17B beamline. These were the transvenous coronary angiography program and the monochromatic-beam computed tomography (CT) program. The programs of DEI and MRT, which are currently active at NSLS, could substantially benefit from the high energy and high intensity of a future wiggler beamline at NSLS-II. In fact, our hopes are to witness the clinical implementation of these two methods at NSLS-II. The following describes in some details the DEI and MRT methods.

Diffraction-enhanced imaging (DEI), an x-ray radiography method that introduces fine selectivity for the angular deviation of x-rays, is considered to be a centerpiece of the medical applications of synchrotron x-rays. DEI, which was developed at NSLS in the late 1990s, uses monochromatic x-rays and an additional monochromator crystal (called the analyzer) between the subject and the detector. The analyzer, with its narrow reflection angular width of a few μradians, provides the ideal tool to analyze the angular distribution of x-rays traversing the subject. The method produces two new type of image contrast. The "refraction" contrast is produced at the interface between two media with difference x-ray refraction indices, while the "extinction" contrast is made when the analyzer crystal rejects the ultra-small angle scattering produced by x-ray diffraction from periodic structures in the tissue. Both enhance soft-tissue imaging, which lacks contrast in traditional x-ray and CT. Besides the DEI research being done at NSLS beamline X15A, the method is being used in essentially every medical research synchrotron beamline around the word.

Microbeam radiation Therapy (MRT) has been one of the major medical research applications of synchrotron radiation that were developed at NSLS. The method studies the effects of irradiation of animal tissues with arrays of parallel, very thin planes of synchrotron-generated x-rays (microplanar beams, microbeams). The highlight of the method is its remarkable tolerance by the normal tissues including the central nervous system (CNS), which has been established at single-fraction doses at as high as several hundred Gy. These beam arrays were also shown to preferentially damage tumors when used at very high doses. Although the underlying mechanisms

of these effects are not well understood, they clearly involve the recovery of both the microvasculature and the glial system. In particular, the microvasculature repair seems to be facilitated by the endothelial and other support cells surviving between the individual microbeams. The method, developed in early 1990s at the NSLS X17B1 superconducting wiggler, also has been pursued since 1996 at the ESRF. The sparing effect of microbeams in the CNS was demonstrated at the NSLS in the adult rat brain and spinal cord, and in the duck embryo's brain, and at ESRF in the cerebellum of suckling rats and piglets. The brain studies at both laboratories used arrays with 27 μm beam width, 50-200 μm beam spacing at up to 625 Gy in-beam doses. Recent studies indicate that microbeams can also be used as a unique tool to study the glial system. Finally, recent studies at the NSLS indicated that the tissue-sparing effect of microbeams stays strong with beams as thick as 0.68 mm. Specifically, two arrays of parallel 0.68-mm-thick planar beams aimed at the target from perpendicular angles were interlaced at the target, producing a non-segmented solid beam at the target while exposing the surrounding healthy tissues to microbeams only.

2. The Growth, Expansion, and Transition of NSLS Scientific Programs

Although the earlier DEI work and much of the present work are being carried out in the planar imaging mode, DEI research is gradually shifting toward CT imaging because the overlying tissue problem of planar imaging does not allow it to take full advantage of the resolving power of DEI. Furthermore, although much of the earlier DEI work used thin samples of small animals (mice and rats), DEI research is going toward the use of larger animals, including rabbits, and thicker tissue samples. The above trends both require higher beam energies and larger beam intensities for which bending magnets fail to be adequate. Therefore, we expect that DEI research at NSLS-II will tremendously benefit from being implemented on a wiggler beamline. Moreover, having a medical wiggler beamline at NSLS-II will make future clinical DEI research in that laboratory possible. As an example, our calculations on the beam flux requirements for DEI CT of a rabbit's head over several slices sing Si (333) reflection and 60 keV energy in about one hour imaging time (necessary to keep the animal anesthetized) indicate that the beam flux from NSLS-II bending magnet will be too low for this purpose by at list two orders of magnitude.

MRT at NSLS-II would benefit from the beam's high energy and high intensity, which are essential for possible clinical studies. In particular, the beamline would allow the use of a heavily filtered beam (e.g., one with a half-power energy of 170 keV to improve dose penetration) at a dose rate of 1,500 Gy/s. This dose rate would allow the delivery of a therapeutic dose (e.g., 150 Gy incident dose) in a fraction of a heartbeat. The current X17B1 beam provides about 40 Gy/s at 120 keV half-power energy. Another possible major advantage of the MRT setup at NSLS-II would be the possibility of a much larger hutch that will allow implementation of MRT in clinical research. For this purpose, the hutch should be adequately larger to accommodate all auxiliary functions such as the physician's control area and medical imaging for subject positioning.

3. Proposed Suite of Beamlines

We propose a dedicated medical application beamline at NSLS-II using a superconducting wiggler. The NSLS-II ring energy at 3.0 GeV and ring current of 500 mA are ideal for both DEI and MRT projects. In fact, higher ring energy is detrimental for these applications. The beamline design should allow future advancements of these two projects to studies with large animals and eventually humans. This includes adequate length and width of the experimental hutches to allow the irradiation hutch together with supportive hutches for a combined medical suite such as those existing at the ESRF, Spring8, and Trieste. Such a concept can only materialize if this beamline is located outside of the right building, and is included in the early stages of the design of the facility. Fortunately, the concept of long beamlines has already been proposed for NSLS-II by other investigators. We believe the long beamline concept can be readily applied to the medical imaging and therapy beamline by locating the facility at the end of the long beamline. We expect a large number of users for both the DEI and MRT projects to join us in the future stages of the proposed beamline design.

ESRF, Spring 8, the Australian and Canadian light sources, and others have all put significant resources recently into dedicated medical beamlines, all using high-energy wigglers. This indicates the great enthusiasm that recent results from DEI and MRT studies around the world has produced in the medical community. More progress is expected at these facilities since most of them are just starting to operate. Trieste has already imaged patients in its mammography suite.

The experimental stations proposed include two side stations utilizing fixed wavelength and two center stations that can utilize both the white beam and focused monochromatic beam. The final experimental and optical requirements would probably lead to a beamline that looks quite different to this in practice.

4. Beamline Specifications and R&D Needs

The highest-energy photons are produced by very high field wigglers. The radiation is broadband with high power at high x-ray energies. The wiggler for the beamline covers the 20 to 150 keV energy range, which requires a critical energy EC of more than 20 keV, corresponding to a wiggler filed of over 3.5 T at the NSLS-II energy of 3 GeV. The brilliance should be as high as possible, which means the wiggler period length should be as small as possible. The combination of high field and small period requires a super-conducting wiggler design. Such devices are available commercially with a peak field of up to 7 Tesla (Weihreter 2004).

A 1 m long superconducting wiggler with 60 mm period and 3.5 T peak field is designated W60 in the NSLS-II CD0 proposal. Its estimated brightness and flux curves are shown in Figures 3.3.3 and 3.3.4 of the proposal. This wiggler provides a 20 keV critical energy, and would serve well as a conservative alternative. To reach a higher critical energy, a wiggler with a higher field of 6 T is proposed.

5. Recommended Transition/Construction Sequence

We recommend transitioning the existing DEI and MRT endstations to the NSLS-II facility from NSLS. Additional expansion of the endstations is being considered.

6. Facility Infrastructure at NSLS-II

The design of the medical suite should include laboratory, animal room, and infrastructures for the future upgrade to a clinical facility.

7. Synergy with other Communities

Brookhaven National Laboratory is an ideal environment for biomedical research. In addition to the current and future synchrotron facilities, there are strong programs in PET, MRI, Cryo-electron microscopy and optical imaging with associated national facilities.