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BER's Role in Biological Research at NSLS-II

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BER-Relevant Programs at the NSLS Today

At the NSLS today, biological and environmental sciences users represent approximately 60% of the user community and more than 2/3 of the facility's publications. They can be broadly categorized into two research communities: structural biology and chemical/structural imaging. The structural biology community focuses on the determination of the atomic structure of biological molecules and complexes. 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.

Uniqueness of NSLS-II

NSLS-II will provide a broadband source of synchrotron radiation from infrared light to x-rays with a brightness unsurpassed by any synchrotron facility worldwide. The extreme brightness and coherence of NSLS-II will enable characterization techniques, such as high-resolution imaging, that are currently in their infancy or do not even exist today. But importantly, NSLS-II will also take widely utilized methods and extend them to new regimes in time- and spatial-resolution that cannot be achieved today.

A Vision for NSLS-II: The Biology Village

NSLS-II plans to follow in the footsteps of the current NSLS by providing a wide range of characterization techniques to the biological sciences community. In January 2008, a series of Scientific Strategic Planning workshops were held at the NSLS to identify a pathway forward to NSLS-II. An overarching conclusion from the Life and Environmental Sciences workshops was the desire within these communities to see increased interaction, collaboration, multi-technique integration, and cross-disciplinary approaches to doing science in the future. It was suggested that this mode of research can be achieved through a "Biology Village" environment, which would include strategically locating beamlines for scientific interaction, having programmatic overlap through shared equipment, technology, and human resources, and playing an active role in the Joint Photon Sciences Institute (JPSI), an interdisciplinary facility at BNL that will facilitate R&D efforts.

Synergies and Partnership between BER and the NIH

BER and NIH have jointly funded a suite of state-of-the-art instruments at NSLS since its beginning a quarter century ago. Now NIH has committed to fund about $45M for at least three insertion device (ID) beamlines at NSLS-II. In June 2009, NIH convened a panel of synchrotron experts to specify characteristics for the initial suite of beamlines. The panel recommended two macromolecular crystallography beamlines, one SAXS/WAXS beamline, and two imaging beamlines, one for soft x-ray coherent diffraction imaging and one for hard x-ray fluorescence microscopy. It is not yet clear that available funds can support all of these beamlines.

Essential Capabilities for BER at NSLS-II

Recent workshop reports by BER have identified both biological challenges and technological needs that are interesting to the BER research community. Most recently, in May 2009, BER held the New Frontiers in Characterizing Biological Systems workshop to address the next generation challenges in genomic science and its connection to functional systems. The panel identified numerous knowledge gaps that inhibit the understanding of biological systems These knowledge gaps are relevant to understanding research areas paramount to BER interest, including the generation and processing of biomass into chemical energy, the cycling of carbon and nutrients, and the transformation of natural and man-made contaminants in the environment.

Synchrotron-based characterization tools are well-suited to fill the identified gaps. Synchrotron studies will generate basic understanding of biological processes, and not just for particular phenomena at a certain physical or temporal scale, but as linked pan-genomically across scales of investigation. With the high brightness and coherence of NSLS-II, structural studies of macromolecules and complexes will be possible in a time-resolved manner, especially in more natural environmental settings. Moreover, high throughput structure/function determination will be able to link genomic information to molecular events. NSLS-II will provide the widest range of nanoscale imaging capabilities in the USA and the Biology Village umbrella will permit multi-modality characterizations of identical samples.

Specifically, NSLS-II envisions a suite of beamlines and associated laboratories dedicated to life and environmental sciences research. We seek co-funding from a range of sources including NIH, DOE-BER, DOE-BES, industrial partners, and foundations. Beamlines will operate in open access to the entire community, irrespective of major funding; BER science will benefit from NIH-funded beamlines and vice versa. In the context of the technology needs established by BER, we include here brief descriptions of proposed beamlines, with a special emphasis on the relevance to programs of BER interest, and the uniqueness based on NSLS-II characteristics.

X-Ray Crystallography on macromolecules (MX) provides the best possible structural information for life science. Undulator beamlines at NSLS-II will allow a push to ever smaller samples, of ever larger complexes. NIH will support one or two initial MX undulator beamlines, but additional capacity is needed. MX coupled with optical spectroscopy is perfect for study of systems matching BER missions. Current studies include metalloenzymes for catabolism of lignin, or chromophores performing photon-sensing and signaling. Such work deserves a three-pole wiggler (TPW) station for in situ specimen characterization. An associated undulator station would serve spectroscopic experiments and also serve other BER-related studies such as gene annotation by high-throughput crystallography, extending JGI efforts to microcrystals.

X-ray scattering is used to retrieve nanoscale shape information of biomolecules and molecular complexes and to detect the subtle changes in their structures. X-ray scattering does not require crystals and can be performed in real biological environments, as biomolecules interact with each other. NIH will support an undulator-based X-ray scattering beamline at NSLS-II to routinely follow biomolecular structural changes on millisecond time scale, such as those that occur in a microfluidic bioreactor as metabolism of biomass takes place or for probing membrane-based biological processes, such as photosynthesis. A TPW beamline is needed for high-throughput static measurements as for genome-wide analyses of cellular components.

X-Ray Footprinting employs a very intense x-ray beam to produce hydroxyl radicals that cause covalent modifications to solvent-exposed regions of nucleic acids and proteins. Using rapid-mixing methods, time-resolved footprinting is used to determine structural changes induced by dynamic processes of large nucleic acid and protein assemblies. With a damping wiggler beamline at NSLS-II, the time resolution will be improved to a microsecond time scale. In addition, shorter x-ray exposure time provides a unique probe of the structure and dynamics of radiation-sensitive membrane proteins, including biological channels that remediate metal atoms in environmental applications and channels that transport ions in photosynthetic bacteria.

X-Ray Absorption Spectroscopy (XAS) is used to measure the speciation, numbers, and types of ligands, and bond distances of ligands surrounding an element. The coupling of a damping wiggler beamline at NSLS-II with advanced detectors and continuous-flow mixing technology will enable XAS measurements at nanoscale concentrations with a time resolution approaching one microsecond. By combining time-resolved XAS and X-ray Footprinting, the relationship between metal coordination and protein conformation can be determined. This is needed, for example, in

dynamic studies of Fe-S clusters in microbe organohalide respiration, metal selectivity in metal-transport proteins, and bacterial ABC transporters. Moreover, focused-beam XAS will create new opportunities for speciation imaging of microbes and rhizosphere chemistry.

Coherent Diffraction Imaging (CDI) is a "lensless" technique for the 2D or 3D reconstruction of nanoscale structures. In CDI, highly coherent x-rays are scattered by the object, producing a continuous reciprocal space diffraction pattern, which is reconstructed into a real space image. An undulator-based beamline at NSLS-II will produce the world's most coherent x-rays for CDI, enabling 3D reconstructions with a spatial resolution as high as 1 – 2 nm. While radiation damage currently limits the resolution to about 10 nm for frozen-hydrated biological samples, this should suffice for important complexes in their natural environment such as membrane proteins, microbial filaments, and lignocellulose nanostructures. NIH seems likely to support a soft x-ray CDI beamline, but there is increasing demand for a hard x-ray CDI beamline.

Transmission X-Ray Tomography (TXM) has been shown to provide insights into the internal 3D structure of whole cells in their natural environment. With recent advances in x-ray focusing optics and the advent of automated cryogenic sample stages, it is possible to collect images from radiation-sensitive cells and to compute reconstructions with a high degree of fidelity to a resolution of 30 nm. In full-field mode, microbe nanostructure can rapidly be imaged in 3D within their cellular communities, whereas scanning (spectroscopic) mode can pinpoint changes in microbe chemistry in response to environmental perturbation.

X-Ray Fluorescence Microscopy is used to image trace element distribution, oxidation state, and ligands within the natural environment of biological cells and tissues. An undulator-based XFM beamline at NSLS-II will be able to routinely generate 3D images with <30 nm spatial resolution and attogram detection sensitivity. Moreover, simultaneous x-ray phase contrast imaging will co-localize trace element chemistry with organelle substructures. Thus, this beamline will be ideal for *in situ* imaging of the chemistry that occurs at the plant-microbe interface such as how endophytic and rhizospheric microbes foster nutrient cycling in biomass production and environmental remediation. BES is funding construction of a general purpose XFM beamline as part of the NSLS-II project but a second branch of this beamline is currently unfunded; whether NIH will support this second branch is not yet clear.

Fourier Transform Infrared (FTIR) Microscopy is used for simultaneously imaging multiple organic components in biological cells and tissues without stains or labels. Since infrared light is non-ionizing, cells can be studied in their natural environment as a function of time. The high brightness and stability of NSLS-II will yield improved spatial resolution (< 1 μm) with a 100-fold increase in detection sensitivity. Coupled to an environmental chamber, FTIR imaging can be used for dynamics studies from microseconds to days on systems including microbe-surface interactions, microbe response to changes in environment, bacterial activity in biofilms, cell exposure to toxins, nutrient cycling in plant-microbe interactions, and biomass catalytic breakdown.