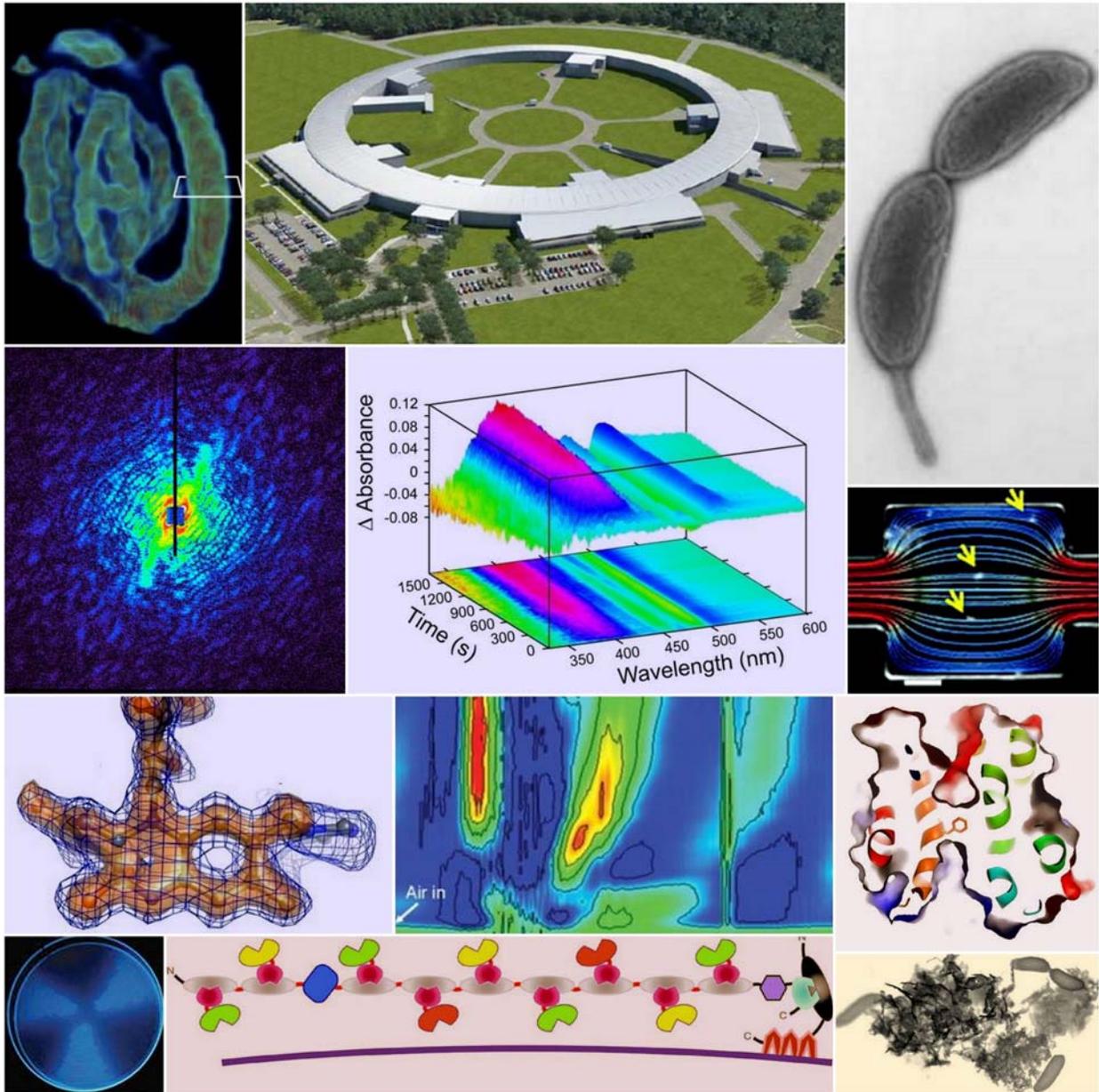


# OPPORTUNITIES AND NEEDS FOR BER SCIENCE AT NSLS-II



January 12, 2010

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## **Contributors**

M. Allaire (BNL)  
M. Adams (Univ Georgia)  
D. Barrick (Johns Hopkins Univ)  
L. Berman (BNL)  
J. Bohon (CWRU)  
Y. Bomble (NREL)  
M. Chance (CWRU)  
J. Chorover (Univ Arizona)  
J. Coates (UC Berkeley)  
F. Collart (ANL)  
F. Colwell (Oregon State Univ)  
B. Dale (Michigan State Univ)  
E. DiMasi (BNL)  
A. Dilmanian (BNL)  
T. Earnest (LBNL)  
S. Gupta (CWRU)  
R. Ferrieri (BNL)  
B. Fox (Univ Wisconsin)  
P. Freimuth (BNL)  
D. Fu (BNL)  
ML Guerinot (Dartmouth Univ)  
R. Hausinger (Michigan State Univ)  
E. Hegg (Michigan State Univ)  
W. Hendrickson (Columbia Univ)  
A. Heroux (BNL)  
R. Hettich (ORNL)  
HY Holman (LBNL)  
C. Jacobsen (Stony Brook Univ)  
J. Jakoncic (BNL)  
A. Lanzirotti (Univ Chicago)  
E. Lima (BNL)  
B. Lindquist (Stony Brook Univ)  
CJ Liu (BNL)  
F. Loeffler (Georgia Tech Res Corp)  
D. Lovley (UMass, Amherst)  
L. Miller (BNL)  
S. Myneni (Princeton Univ)  
P. Northrup (Stony Brook Univ)  
A. Orville (BNL)  
G. Phillips (Univ Wisconsin)  
T. Punshon (Dartmouth Univ)  
H. Robinson (BNL)  
D. Salt (Purdue Univ)  
D. Schneider (BNL)  
R. Scott (Univ Georgia)  
J. Shanklin (BNL)  
D. Shapiro (BNL)  
Q. Shen (BNL)  
W. Shi (CWRU)  
P. Sobecky (Univ Alabama)  
A. Soares (BNL)  
V. Stojanoff (BNL)  
M. Sullivan (CWRU)  
J. Sutherland (BNL)  
R. Sweet (BNL)  
R. Tappero (BNL)  
J. Tainer (LBNL)  
J. Thieme (BNL)  
N. van der Lelie (BNL)  
J. Wall (Univ Missouri)  
J. Walton (Michigan State Univ)  
G. Waychunas (LBNL)  
D. Wildenschild (Oregon State Univ)  
S. Wirick (Stony Brook Univ)  
D. Wu (Univ Rochester)  
L. Yang (BNL)  
Z. Zhong (BNL)

## **Executive Summary**

The National Synchrotron Light Source II (NSLS-II) will provide synchrotron radiation of unparalleled brightness and stability across the entire spectral range from the infrared to high-energy x-rays. NSLS-II will begin operations in 2015, replacing the existing NSLS. The extreme brightness and coherence of NSLS-II will enable new techniques, such as nanoscale imaging, that are currently either in their infancy or not feasible with present sources. Moreover, NSLS-II will take widely used characterization techniques, such as macromolecular crystallography (MX), X-ray scattering (XRS), and X-ray absorption spectroscopy (XAS) and extend them to regimes in time- and spatial-resolution that cannot be achieved today.

The extraordinary capabilities of NSLS-II present many new research opportunities for biology and environmental science. BER-supported scientists and others have described such research as can be envisioned at this time in the three broad areas of bioenergy solutions, carbon cycling and biosequestration, and contaminant transport and cleanup in the environment; and these and other prospective science opportunities at NSLS-II are summarized in this document.

Although beamlines that are already planned for NSLS-II with support from DOE-BES and NIH will also be available for BER-supported applications, many beamline needs for BER science remain unmet at present. BER science opportunities have been considered in light of the unique NSLS-II capabilities, and the characteristics of beamlines that can support this science have been elaborated. All of these beamlines will ultimately be needed to pursue the science opportunities outlined here.

## Chapter 1: Overview and Uniqueness of NSLS-II

The National Synchrotron Light Source II (NSLS-II), which is now under construction at Brookhaven National Laboratory, will provide a broadband source of synchrotron photons from infrared light to X-rays with a brightness unsurpassed by any synchrotron worldwide. This new facility is scheduled to be operational in 2015 and will replace the existing NSLS.

The extreme brightness and coherence of NSLS-II will enable new techniques, such as nanoscale imaging, that are currently either in their infancy or not feasible with present sources. Moreover, NSLS-II will take widely utilized characterization techniques, such as macromolecular crystallography (MX), X-ray scattering (XRS), and X-ray absorption spectroscopy (XAS), and extend them to new regimes in time- and spatial-resolution that cannot be achieved today.

At NSLS today, biological and environmental sciences users represent approximately 60% of the user community and more than 650 publications annually. BER funding has played a critical role in this success by funding state-of-the-art programs since the beginning of NSLS over a quarter century ago. NSLS-II plans to follow in the footsteps of the existing NSLS by providing a wide range of characterization techniques and expertise to the biological sciences community.

Recent workshops by BER have identified both biological challenges and technological needs that are important to the BER research community. For example, in May 2009, BER held the New Frontiers in Characterizing Biological Systems workshop to address the next generation challenges in genomics 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, climate change and 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 a wide range of nanoscale imaging capabilities, permitting multi-modality characterization of identical samples.



*NSLS-II will be operational in 2015 and will provide a broadband source of photons with a brightness and coherence unsurpassed by any synchrotron worldwide. For BER science, it will enhance time-resolved structural studies of macromolecules and complexes, especially in more natural environmental settings. High throughput structure/function determination will be optimized to link genomic information to molecular events. And it will provide a wide range of nanoscale imaging capabilities with the possibility of multi-modality characterization of identical samples.*

In early 2008, a series of Scientific Strategic Planning workshops were held by NSLS and NSLS-II 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. The idea arose that this mode of research can be achieved through a “Biology Village” environment, which would include strategically locating beamlines for scientific interaction and taking advantage of programmatic synergies through shared equipment, technology, and human resources.

NSLS-II envisions a suite of beamlines and associated laboratories dedicated to life and environmental sciences research. Funding from a range of sources, including NIH, DOE-BER, DOE-BES, industrial partners, and foundations, is needed to realize this. BER’s expertise in promoting new opportunities for science at the interfaces between these scientific fields can serve as a blueprint for such a village concept. Access to all beamlines will be open to the entire community and based on merit, irrespective of funding source. In this way, BER science will benefit from beamlines constructed and operated with NIH funds and vice versa.

BER and NIH have jointly funded beamlines at NSLS for many years. The BER/NIH-funded Macromolecular Crystallography Research Resource (PXRR) has an especially strong record, supporting experiments that include research recognized by Nobel Prizes to Roderick MacKinnon in 2003 and to Venkatraman Ramakrishnan and Thomas Steitz in 2009. In June 2009, NIH convened a panel of synchrotron experts to specify characteristics for an initial suite of beamlines. The panel recommended two MX beamlines, one XRS beamline, and two imaging beamlines, one for soft X-ray coherent diffraction imaging and one for hard X-ray fluorescence microscopy. NIH has committed to provide about \$45M in funding to construct four insertion device (ID) beamlines at NSLS-II, including two MX beamlines, one XRS beamline, and one imaging beamline, and \$12M is already being transferred to NSLS-II for inside-the-shield-wall components. The characteristics of the imaging beamline are yet to be determined.

**Abbreviations used:**

CDI: coherent diffraction imaging  
FTIRM: Fourier transform infrared microscopy  
ID: insertion device  
MX: macromolecular crystallography  
PXRR: Macromolecular Crystallography Research Resource  
SAXS: small-angle X-ray scattering  
STXM: scanning transmission X-ray microscopy  
WAXS: wide-angle X-ray scattering  
XAS: X-ray absorption spectroscopy  
XFM: X-ray fluorescence microscopy  
XRS: X-ray scattering  
XFN: X-ray fluorescence nanotomography

In this document, we describe how the unique characteristics of NSLS-II can be used to address scientific challenges particularly interesting to BER researchers, specifically a systems-level understanding of plants and microbes in bioenergy solutions, climate and carbon cycle, and contaminant transport and cleanup. We focus on unanswered scientific questions (Chapter 2) and describe a suite of beamlines (Chapter 3) that will be able to address these challenges in ways not possible today by taking advantage of the unique characteristics of NSLS-II.

## **Chapter 2: Science Opportunities**

### Introduction

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The fundamental research goal of the [BER Genomics: GTL program](#) is to “achieve a predictive, system-level understanding of plants, microbes, and biological communities, via integration of fundamental science and technology development, to enable biological solutions to DOE mission challenges in energy, environment, and climate.” One of the primary objectives of this goal is to “develop the experimental capabilities and enabling technologies needed to achieve a genome-based, dynamic system-level understanding of organism and community functions.”

In this chapter, we describe how scientific problems relevant to BER scientists can be addressed by the unique characteristics of NSLS-II, by presenting a series of applications to bioenergy, carbon cycling and sequestration, and contaminant transport and cleanup in the environment. We emphasize a range of techniques that will enable multi-scale exploration: (1) at the molecular level, to understand how genes determine biological structure and function, (2) at the cellular level, to understand how molecular processes are coordinated to execute cell function, and (3) at the level of microbial communities and higher organisms to understand how cells interact and respond to their environment. Here we present just a few examples that illustrate the breadth, excitement, and importance of the advances enabled by NSLS-II.

For example, the NSLS-II facility will:

- enable the determination of atomic resolution structures of macromolecular complexes involved in biofuel production, light harnessing, and contaminant cleanup
- facilitate dynamics measurements with the time resolution necessary to fully characterize new enzymes and complex assemblies in order to understand their functions as “macromolecular machines”
- provide a range of nanometer-resolution probes to image the complex structures and molecular chemistry of lignocellulosic biomass, microbes and microbial communities, and microbe-plant interfaces
- make available high-throughput technologies for rapidly correlating genomics with structural and functional information on macromolecular complexes

### Bioenergy Solutions

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Biofuels derived from cellulosic plant biomass – the fibrous or woody plant materials such as stems and leaves – have the potential to provide a secure, renewable source of energy that will reduce our dependence on fossil fuels, and our net emission of greenhouse gases ([DOE-BER Biofuels Strategic Plan](#)). Importantly, biofuels derived from cellulosic feedstocks do not directly compete with food resources and can be grown on lands unsuitable for traditional food agriculture. Moreover, “designer” biomass feedstocks have the potential for much greater yields of cellulosic material, decreased requirements for water or fertilizer, and greater tolerance to pests and disease.

Despite these advantages, cellulosic biofuels are not yet widely available because their biomass is a complex, heterogeneous material that is difficult to degrade into its component sugars. As such, cellulosic biofuels are currently not cost-competitive with either fossil fuels or ethanol produced from corn starch. In addition, ethanol has considerably lower energy density and is more difficult to distribute via existing infrastructure than gasoline. Thus, significant technical barriers need to be addressed before cellulosic biofuels can be more broadly adopted for use.

As described in the [Genomics:GTL Strategic Plan](#), systems-biology approaches for designing and engineering bioenergy plant and microbial systems will generate knowledge of the mechanistic bases for key bioenergy challenges:

- Produce high-yield sustainable biomass crops with designer lignocellulosic composition
- Consolidate processes to use one microbial species or community for both degradation and fermentation
- Produce biofuels beyond ethanol to improve energy density, processing, and handling

In order to address the complexity of these challenges and achieve industrial-scale bioenergy production, the DOE established three [Bioenergy Research Centers](#) in 2007. We will work with these teams and individual researchers to address these challenges using synchrotron-based characterization methods. Some are highlighted below.

### **Lignocellulose structure, composition, and breakdown**

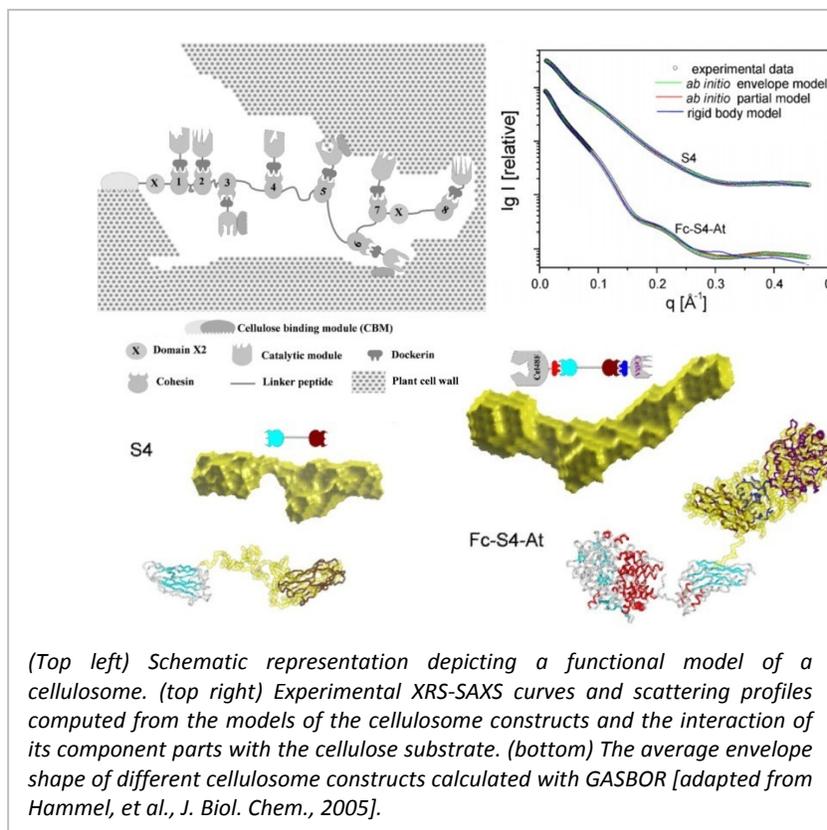
In order to develop optimized biomass crops, we need to understand further how enzymes interact with cellulose to maximize enzymatic degradation. One effort to lignocellulose deconstruction involves microbial enzymes, including cellulases and hemicellulases, which degrade the microcrystalline cellulose structure. Our knowledge of these deconstructive enzymes has been advanced recently by the Genomes to Life sequencing effort.

One approach is to improve the thermal stability of these enzymes by utilizing hyperthermophilic organisms. For example, *Calidcellulosirupor* is a group of extreme thermophilic bacteria that are known to ferment polymeric carbohydrates at high rates. The integrated genome and proteome datasets identified the presence of highly active cellulolytic enzymes and revealed their unusual thermostability. Mass spectrometric analysis has been used to identify numerous multi-protein complexes, but provide no structural details. If crystals can be obtained, they are often small and fragile. Furthermore, diffraction from such crystals is generally weak, with successful data collection often requiring the intensity, small beams, and high coherence of a third-generation synchrotron source. Indeed, one key advantage that NSLS-II brings to the global MX community will be the ability to determine structures from microcrystalline samples, such as those enzymes that catalyze the critical transformation steps in lignocellulose breakdown.

To understand enzyme function, one must understand the intricacies of multi-domain proteins, such as carbohydrate-binding domains and the catalytic domains (i.e. how these are assembled and how they interact). Solution X-ray scattering can rapidly determine the nanoscale structures of these complexes and X-ray footprinting can provide time-resolved structures at atomic-resolution. For both techniques, the high brightness of NSLS-II will permit the use of microfluidic flow cells to reduce sample volumes and improve time resolution from milliseconds to microseconds or better for dynamical studies.

A combination of the GTL sequencing effort and biochemistry has demonstrated that, although these enzymes are rather slow on their own, nature has greatly enhanced their reactivity by binding them together in large multi-enzyme collections known as “cellulosomes”. These extracellular complexes contain both cellulose- and hemicelluloses-degrading enzymes as well as cellulose-binding modules. By gathering multiple enzymes of different activity and specificity together on the substrate, considerable synergy through proximity has been achieved. Again, a molecular level understanding of the structure and function of cellulosomes that can be obtained from MX, XRS, and footprinting is necessary for improving their efficiency, and perhaps for designing artificial cellulosomes for large-scale biofuels production in the future.

In addition to understanding the molecular-level structure of these enzyme systems and how they relate to function, the next step in a systems-biology approach is to understand the location and interactions of these molecules at the cellular level. Currently we have a limited understanding of the structural properties of plant cell walls that impart strength and resistance to degradation, hindering development of better strategies for biomass deconstruction. For example, lignin, an abundant, highly cross-linked, largely aromatic biopolymer, greatly reduces the availability of cellulose for conversion into fermentable simple sugars, which therefore lowers the efficiency of converting biomass to ethanol or other biofuels. Lignin monomers are produced in the cytosol and transported into the cell wall for polymerization.



(Top left) Schematic representation depicting a functional model of a cellulosome. (top right) Experimental XRS-SAXS curves and scattering profiles computed from the models of the cellulosome constructs and the interaction of its component parts with the cellulose substrate. (bottom) The average envelope shape of different cellulosome constructs calculated with GASBOR [adapted from Hammel, et al., J. Biol. Chem., 2005].

Nanoscale imaging is required to visualize the formation of the lignocellulosic network. For example, diffraction-based X-ray imaging can be used to determine the structure of crystalline cellulose and soft X-ray spectromicroscopy can assess the chemical effects of enzymatic degradation. Additionally, synchrotron infrared imaging can provide images of the real-time degradation of cellulose at submicron-scale resolution.

### Improving biomass yield

Recent studies by BER researchers have shown that endophytic bacteria in the environment of the plant root can enhance the rate of Poplar biomass production. However, very little is known about the mechanism of plant growth promotion or about the symbiotic relationship between the plant root and the bacteria. Nanoscale X-ray imaging provides a nondestructive means to study the structure and transport of materials at the plant-microbe interface and to glean unique *in situ* chemical and spatial information that is crucial for evaluating physiochemical changes in heterogeneous systems at ambient (environmentally-relevant) conditions.

One particular issue for biofuels feedstock production on marginal lands (i.e. poor soil fertility) is to understand how endophytic bacteria assist plants in coping with nutrient-limited soil conditions (e.g. Fe deficiency). Nanoscale X-ray imaging is required to investigate nutrient cycling and flux in the Poplar/endophyte rhizosphere microenvironment (plant-microbe-soil interface). X-ray fluorescence imaging and nanotomography are used to study the 2D and 3D distributions and elemental associations on the

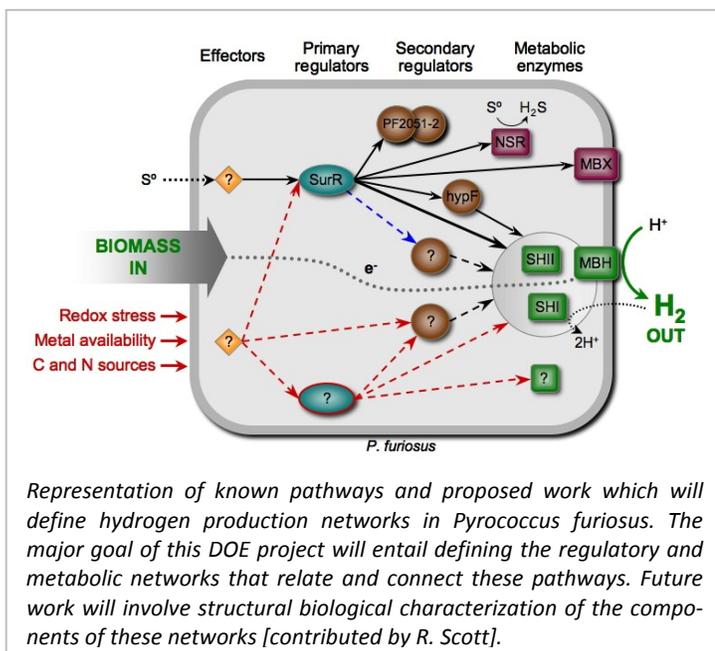
(sub)micron scale, and X-ray absorption spectroscopy (XAS) is used to determine the molecular speciation of a target element (e.g. Fe) at each pixel of an X-ray fluorescence image.

With the capabilities of NSLS-II, researchers will be able to observe these biogeochemical processes for the first time at the sub-cellular level, providing new information on the mechanism of plant growth promotion. Importantly, the high brightness of NSLS-II, and the advanced X-ray detectors being developed at NSLS, will provide unsurpassed spatial resolution along with rapid data collection rates. A wide range of root-microbe systems can be examined quickly.

### Alternative energies beyond carbon-based biofuels

Microbes are not only capable of producing carbon-based biofuels, but they are also well-known as efficient sources of alternate energy. For example, the thermophilic *Pyrococcus furiosus* has the potential to produce hydrogen ( $H_2$ ) efficiently from biomass, making it an excellent target organism for development of this alternative energy source. The SAPHyRe project is aimed to develop a detailed systems-level description of the regulatory and metabolic networks controlling hydrogen production in this organism. It will also be used as a model organism to investigate its response to various environmental conditions relevant to all hydrogen-producing microorganisms, such as carbon and nitrogen sources, metal availability, and oxidative and reductive stresses.

*Clostridium thermocellum* is another thermophilic bacterium that evolves hydrogen at a high rate during cellulose degradation. Analysis of its genome sequence reveals at least 20 genes are potentially related to hydrogen metabolism. The bacterium is thus remarkably versatile in employing various enzymes, some of which are potentially novel, for hydrogen metabolism. To have a thorough understanding of this biological process could have an impact in bioenergy endeavors. Yet little is known concerning the pathway for hydrogen production and the regulatory mechanism/network that control these hydrogen genes, or about the cellulolytic process or other metabolic pathways in this organism.



In all microbial systems studied to date, the enzymes that catalyze the production of hydrogen ( $H_2$ ) from  $2H^+$  and two electrons are metalloenzymes. Not surprisingly then, several of the Bioenergy Research Centers employ experts in metalloprotein catalysis. Their interests involve using photons from the sun to drive  $H_2$  and/or methane ( $CH_4$ ) production.

One needs a better understanding of the factors that control charge separation and influence the electron transfer pathways of the metalloenzymes involved to achieve these goals. A focus of the Bioenergy Research Centers is *bio-prospecting* for new microbes and enzymes to realize the goals of bioinspired  $H_2$  and/or  $CH_4$  production. Excellent sources for these new enzymes are the microbes and

metagenomes isolated from terrestrial hot springs and deep sea hydrothermal vents, because the enzymes will be stable.

The energy source of these ecosystems is from mid-ocean ridge volcanism found at black smokers and in the off-axis vents supporting the carbonate chimneys of the “Lost Cities”. They harbor the oldest microbial life forms with biochemical metabolism based upon H<sub>2</sub>-dependent and C<sub>1</sub>-dependent chemistry driven by transition-metal sulfide catalysts. Consequently, understanding the structures and functions of many diverse metalloproteins is essential to several strategic aims of the Bioenergy Research Centers. Correlated MX and single-crystal spectroscopic analysis capabilities, along with XAS and fluorescence nanotomography, enabled by NSLS-II are well-suited for these types of macromolecules.

The ability to reduce a wide variety of metals including first row transition metals like iron and manganese, to uranium, technetium, and even gold, is a highly conserved characteristic of hyperthermophilic Archaea and bacteria. This has led, in part, to the recent discovery of a new form of microbial respiration in which microorganisms support growth by completely oxidizing organic compounds to carbon dioxide commensurate with direct quantitative electron transfer to external terminal electron acceptors. These “microbial fuel cells” offer the possibility of harvesting over 90% of the electrons from organic renewable biomass in the form of electricity.

Structural studies coupled to single-crystal spectroscopy will provide a better understanding of electron transfer processes and should aid in predicting and designing for bioremediation of aquatic sediments and a variety of other waste organic materials. In addition, nanoscale resolution and chemical sensitivity using X-ray fluorescence nanotomography will help establish the microbe-mineral interactions and their influences on microbial fuel-cell efficiency. The nanoscale spectroscopic imaging capabilities of these beamlines will provide researchers the ability to directly image the tremendous array of widely varying metabolic processes the organisms catalyze. FTIR imaging will provide spatially-resolved dynamics information on the distribution and mineral species formed in these surface interactions.

In all cases, the structural and functional characterization of the critical molecular players identified through these systems will enable a detailed understanding of the hydrogen production process and its regulation. A variety of structural characterization techniques are poised to aid in this endeavor, including X-ray crystallography, small-angle X-ray scattering (SAXS) and X-ray footprinting. In addition, since many key enzymes in the process are metalloenzymes, bulk and nanoscale extended X-ray absorption fine structure (EXAFS) and X-ray absorption near edge spectroscopy (XANES), and MX with correlated optical spectroscopy studies can be used to interrogate the metal-binding active sites in critical detail.

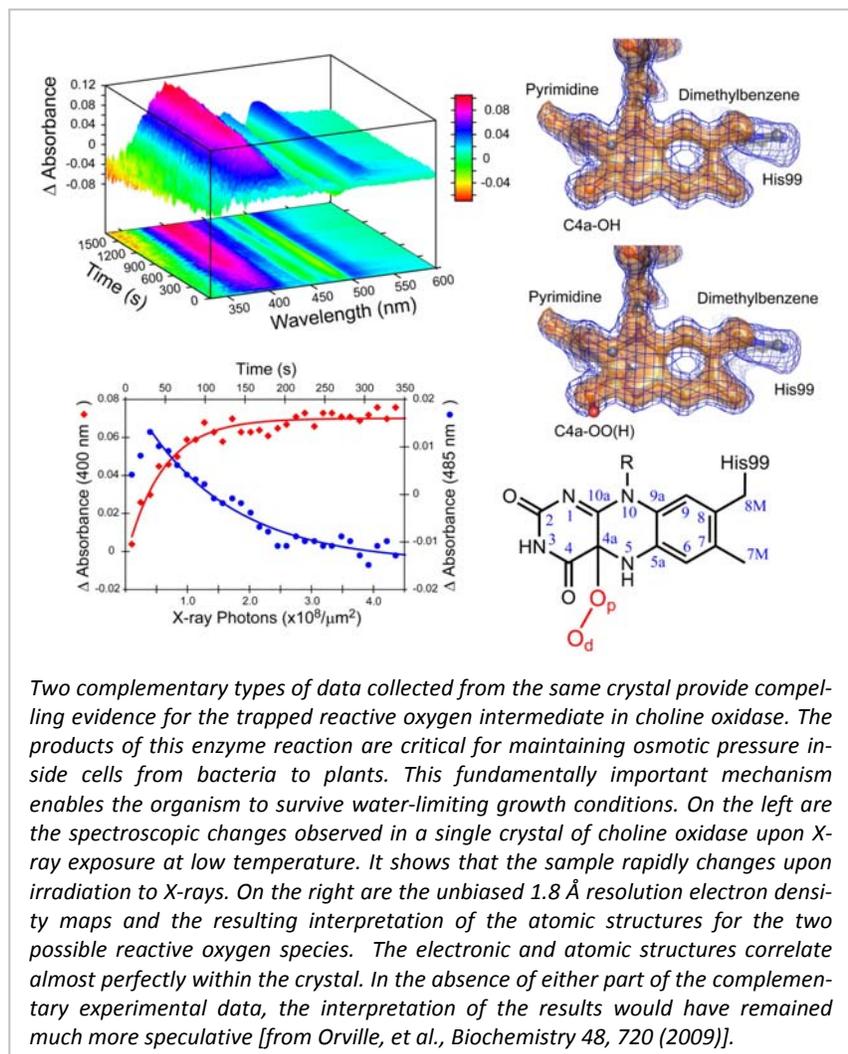
One particular issue for microbial production of molecular hydrogen is to understand the plethora of Fe-S proteins involved in the process. Many putative proteins are simply annotated as Fe-S proteins without a hint of function. These types of biological samples are often quite low in concentration, requiring the significant flux density and/or high brightness X-ray beams and extremely sensitive detection capabilities of NSLS-II in order to acquire high quality data.

The transmembrane movements of metal ions often occur in milliseconds, while many metal-exchange reactions occur in hours or days. How these energetic and kinetic properties are realized in metal transporters is not clear, especially considering the high metal mobility contrasted with strict metal selectivity. X-ray footprinting will help to characterize the kinetic process of metal transport on the millisecond timescale, and X-ray crystallography with correlated spectroscopic analysis should reveal the molecular architecture of metal transporters in atomic detail. We will also develop *in situ* molecular imaging of chloroplast membrane proteins to place these studies in a biological context related to the chloroplast biogenesis and function.

Fatty acids are primary constituents of membranes and of storage lipids, with over 1000 distinct examples found in nature. They are ideal for energy storage in seeds because they have an energy density eight-fold greater than that of starch, approximately that of petrochemicals. Saturated fatty acids are most often converted into unsaturated fatty acids by iron-dependent metalloenzymes.

The majority of fatty acid diversity results from the action of O<sub>2</sub>-dependent fatty acid desaturases and related enzymes that consist of two independent enzyme classes; one soluble, one membrane bound. Combined physical biochemical / structural approaches are being used to correlate reaction-cycle intermediates with crystal structures. Moreover, lipids have unique FTIR spectroscopic signatures that can be used to correlate genomics information with enzymatic reaction products in these systems.

The structural bases for regioselectivity and reaction outcome are under active investigation; because control of these properties will enable the design of enzymes to produce novel renewable chemical feedstocks and biofuels with improved properties. Work on the crystal structures of the desaturase-Acyl Carrier Protein complex and the first crystal structure of an integral membrane desaturase are ongoing, but extremely challenging. Essential tools for these ongoing studies at NSLS-II will be X-ray footprinting, correlated single-crystal spectroscopy with MX, and S/WAXS studies.



## Carbon Cycling and Biosequestration

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There is currently a strong scientific consensus that increasing concentrations of greenhouse gases in the atmosphere are the chief cause of recent global warming and accelerated sea level rise, and that the carbon dioxide (CO<sub>2</sub>) emitted from combustion of fossil fuels is the most important source of growing greenhouse gas concentrations. Both terrestrial and ocean microbes cycle immense volumes of carbon in the process of recycling the Earth's biomass. They are the primary photosynthetic producers of biomass in the ocean and act as carbon managers and decomposers in terrestrial systems. They can fix CO<sub>2</sub> by light and geochemical reactions; they can generate methane; they can produce CO<sub>2</sub> as they decompose organic matter; they can precipitate carbonate minerals; and they can catalyze the polymerization of plant polymers into recalcitrant pools of carbon in soil ([Genomics: GTL Strategic Plan, 2008](#)).

As described in the [Genomics: GTL Strategic Plan](#), a major mission of BER research programs focuses on the linkage of global biogeochemical processes to genome-based biological functions of plant and microbial communities to address key carbon cycling challenges:

- develop a mechanistic understanding of carbon cycling in the Earth's marine and terrestrial ecosystems
- understand and optimize the biological sequestration of carbon
- determine how climate change affects biological processes that influence carbon cycling and biosequestration

In order to address the complexity of these challenges, NSLS-II can provide analytical advances to understand how marine and terrestrial microorganisms fixate and cycle carbon and how these processes affect climate change. Some examples are highlighted below.

### **Carbon sequestration as carbonate minerals**

By stimulating urea hydrolysis, certain microbes have been shown to sequester carbon by precipitating calcium carbonate mineral. These microbes are also of interest because they simultaneously immobilize <sup>90</sup>Sr through co-precipitation. Model studies at different laboratory scales are being used to visualize and quantitatively describe the spatial relationships between amendment transport and consumption that stimulate the production of biomass and mineral phases.

The growth, evolution, and distribution of microbial activity and mineral formation are important for optimizing biosequestration and can be uniquely elucidated with high resolution and chemical sensitivity using synchrotron X-ray and infrared imaging. This is critically needed due to the heterogeneity present in natural environments. Nanoscale imaging techniques offered by X-ray probes will allow researchers to isolate and identify strategically located micro-niches within natural aggregates which aid the sequestering of carbon. Advances in X-ray tomographic imaging now allows us to structurally examine intact soil aggregates and undisturbed soil volumes 3-dimensionally with spatial resolutions as small as 2-3 microns. However, next generation tomography probes at NSLS-II will allow for in-situ, three-dimensional spectroscopic chemical imaging of candidate carbon-sequestration materials within experimental cells at both X-ray and infrared beamline. This will allow BER funded scientists to quantify the convection/dispersive flow patterns of abiotic and biotic materials through selected soil and sub-surface media. This will provide powerful new insight into how the chemistry of the interior surfaces of pores contributes to both the accessibility and movement of soil micro-organisms.

## Environmental Genomics

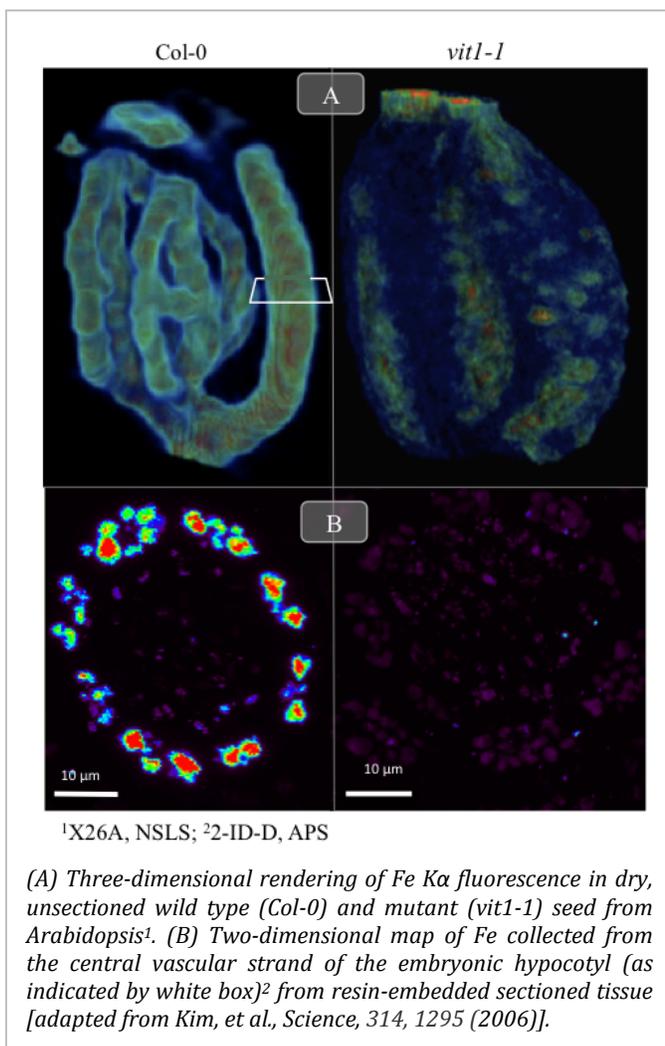
Opportunities to examine how genetic variations in organisms affect their interactions with the environment are driven by the rapidly increasing quantity of nucleotide sequence data that has been provided by the GTL program. Synchrotron-based X-ray imaging techniques are extremely well suited to aid in the evaluation of how specific genes influence the uptake of nutrients and contaminants in plants. This is done by imaging these interactions at the subcellular level, yielding non-destructive, three dimensional characterization of elemental distribution potentially *in-vivo*.

These types of studies require the application of high-throughput elemental analysis technologies and their integration with both bioinformatic and genetic tools. For example, biofortification involves developing plants that store higher concentrations of bioavailable nutrients in the seed. To do this, functions of the ion transport and storage genes need to be known. Our ability to characterize the function of genes involved in elemental homeostasis would be rapid and comprehensive; identifying the organelle in which a particular element accumulates would allow us to rapidly target the genes responsible.

## CO<sub>2</sub> levels, carbon fixation, and photosynthesis

Plants, including trees, sense and respond to increasing CO<sub>2</sub> through increased photosynthesis and reduced stomatal conductance. However, genetic and environmental bottlenecks can determine both the magnitude of these primary responses to CO<sub>2</sub> and the capacity to assimilate carbon into increased above-ground biomass. Although the responses of photosynthesis, growth and biomass accumulation in trees grown at elevated CO<sub>2</sub> are well documented, the molecular mechanisms that determine how different tree species achieve a balance between carbon and nitrogen assimilation, storage and eventual growth remain largely unknown. Consequently, there is a need to increase our understanding of metabolic/physiological processes that may limit the response of trees to increasing CO<sub>2</sub>. For example, structural knowledge of the membrane proteins involved in stomatal pores in the leaves of plants is necessary to understand the influx of atmospheric carbon dioxide in exchange for transpirational evaporation of water.

Chloroplasts are one of the most metal-enriched organelles in life systems. Metals are present in chloroplasts at millimolar levels; most are protein-bound, while unligated metals are toxic in aerobic environments. Consequently chloroplasts maintain delicate control of metal



concentration. Plants employ metal-transport proteins to acquire metal ions from the soil and then other transporters in the chloroplast maintain appropriate free-metal concentrations. Many essential metal ions are scarce in the soil, so transporters must pump them across the membrane barrier against concentration gradients.

NSLS-II nanoimaging beamlines will allow researchers new innovative tools by which to quantify integrated responses of plants for fixing atmospheric CO<sub>2</sub>. Coupled with radioisotope-tagged PET imaging, these instruments will allow scientists to visualize photosynthetic pathways involved in photosynthesis, soil nutrient assimilation and transport during stages of growth, and to understand how those pathways might change in response to environmental conditions (such as elevated CO<sub>2</sub> and ozone, drought). For example, these instruments may allow us to visualize *in-vivo* DAHP synthase mediated cellular modifications in Mn<sup>+2</sup> and Co<sup>+2</sup> DAHP synthase activity. DAHP is a key enzyme providing substrate for lignin biosynthesis.

## Contaminant Transport and Cleanup in the Environment

DOE is committed to remediating the large volumes of soil, sediments, and groundwater contaminated with metals, radionuclides, and a variety of organics at diverse defense facilities and sites across the nation. In situ bioremediation, taking advantage of natural microbial populations in the subsurface, has the potential for reducing costs and increasing the efficiency. DOE-BER bioremediation strategies and biogeochemistry research focus on using natural microbial communities to reduce the mobility and toxicity of metals and radionuclides. The interdependent metabolic survival strategies used by microbial communities can directly or indirectly remove contaminants from groundwater or transform toxic contaminants into benign chemical products.

A biotreatment technique that works well at one site may perform poorly at another because microbial communities, geochemical properties, and flow regimes frequently differ markedly between sites. We often lack understanding of how microbial processes are coupled to other processes influential in contaminant behavior and are scaled in heterogeneous environments. In addition, we need new tools for measuring key microbial, geochemical, hydrological, and geological properties and processes in these systems. Less than 1% of all microorganisms collected at only a few sites have been cultured and characterized in any great detail, and only a small fraction of those have been sequenced. Even less is known regarding the interactions of microorganisms in communities ([Genomics: GTL Strategic Plan](#)).

As part of the [Genomics: GTL Strategic Plan](#), it will develop methods to relate genome-based understanding of molecular processes to long-term conceptual and predictive models for simulating contaminant fate and transport and development of remediation strategies. Challenges for GTL include:

- Understand the complex interactions of microbes with contaminants and the subsurface environment
- Develop new suites of biosensors and performance-assessment tools and analyze natural microbial communities' functions using genome-based, multidisciplinary, field-oriented approaches to generate a mechanistic and predictive understanding of microbial responses to contaminants or nutrients
- Characterize biogeochemical processes from the fundamental molecular to community level to describe contaminant-transformation processes coinciding with simulated changes in microbial community composition and structure

By combining genomics with structural and mechanistic studies, NSLS-II can provide a range of new tools that generate a molecular level understanding of these processes with a goal of developing long-term models for predicting the transport and fate of contaminants in order to produce viable remediation strategies. Some examples are highlighted below.

### **Microbe-mineral interface in contaminated environments**

Interactions at the microbe-mineral interface in soils and sediments can influence contaminant behavior. The GTL program has identified a number of microbial systems that have the potential to remediate environmental contaminants. For example, *Shewanella* and *Geobacter* – two GTL model systems – can enzymatically transform Uranium(VI), which is soluble and moves in groundwater, to Uranium(IV), which is insoluble and precipitates out of the groundwater as a biologically unavailable solid. Conversely, recent studies have indicated that microorganisms capable of bio-oxidation of U(IV) inhabit uranium-contaminated and uncontaminated DOE sites. These organisms, including strain TPSY and “C. millennium”, can utilize U(IV) as the sole electron donor which has the potential to produce mobile U in anoxic environments. In order to predictably model remediation efforts based on U(VI) reduction, it is essential to understand the microorganisms and the physiology of anaerobic, U(IV) bio-oxidation and the impact of this microbial metabolism on the long term sequestration of U in the environment.

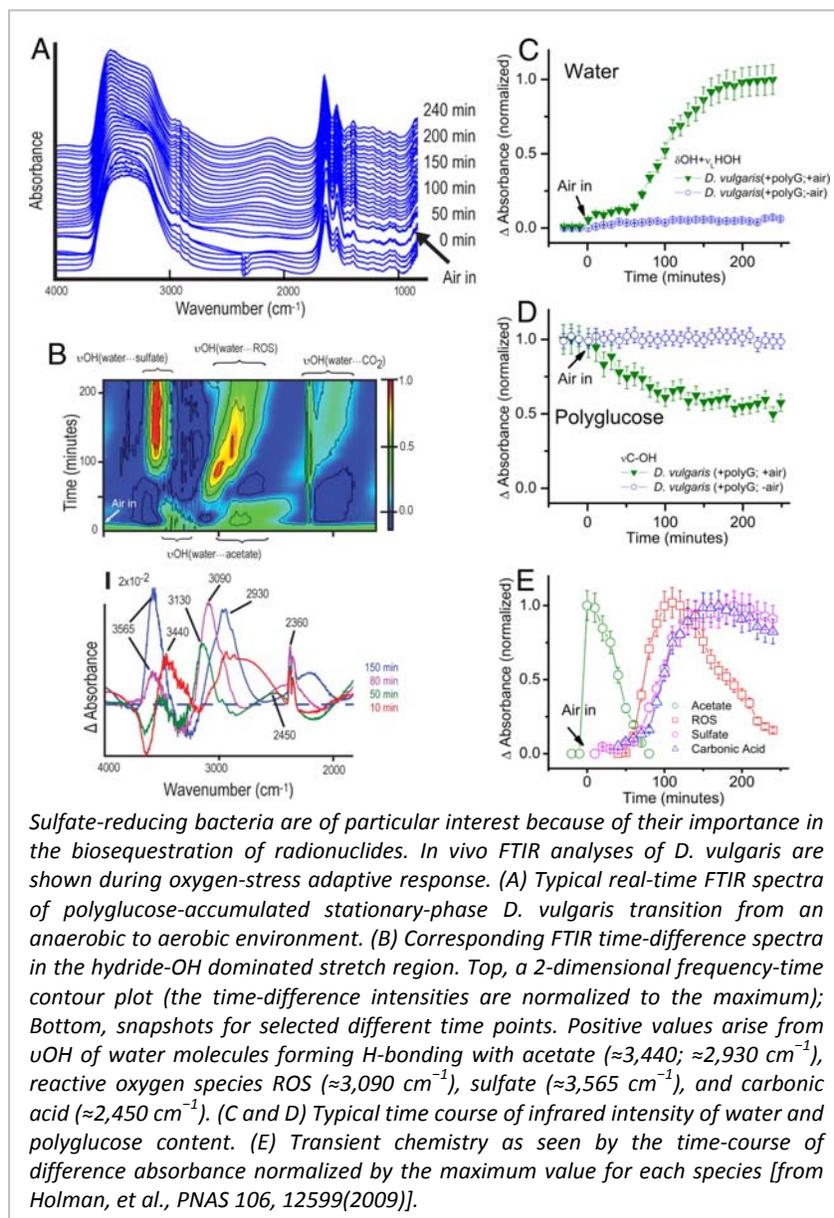
Other studies have shown that intrinsic phosphatase activities of indigenous subsurface microbes result in the release/accumulation of sufficient phosphate to cause the formation and precipitation of low solubility U-phosphate minerals in oxygenated groundwater and soil. The proposed biotransformation, with its emphasis on an aerobic process, can be considered to serve as a secondary biobarrier strategy for U immobilization should the metal precipitates formed by dissimilatory mechanisms remobilize due to a change in redox state. Phosphate mineral formation and distribution can be monitored *in-vivo* using micro- and nano-scale FTIR and X-ray diffraction imaging and correlated with X-ray fluorescence imaging of the uranium distribution and speciation.

To understand the mechanisms behind these microbe-mineral interactions, studies at different laboratory scales are necessary. Available genome information and U(VI)-reducing isolates will be used to study fundamental, molecular-level processes and understand cellular, population and community level interactions, functions and dynamics. Culture-based studies define and compare the nutritional and environmental requirements of U-reducing bacteria. DNA-, RNA-, and proteome-based tools can be used to monitor the presence, abundance, dynamics, spatial distribution and activity of target organisms in the contaminated subsurface. Multi-collector-ICPMS provide high precision U isotope measurements to quantify U reduction reactions and provide in situ rate information. The new qualitative and quantitative tools will be tested, refined and validated in continuous flow columns and synchrotron-based techniques (XANES and XAFS) will confirm U redox state and complexation. Community-encompassing systems understanding provided by this comprehensive set of tools supplies the technical framework for selecting the most promising and cost-effective remediation technology.

### **Bioremediation coupled with carbon sequestration**

Although it is commonly accepted that bioremediation holds great promise for dealing with intractable environmental problems, a significant amount of work remains to be done to fully realize the potential of bioremediation. Laboratory and *ex-situ* experiments have shown that microorganisms can change the valence and oxidation state of certain heavy metals and radionuclides by using them as elec-

tron acceptors and thereby alter either reactivity and/or mobility of the contaminants in the subsurface. In addition, many microorganisms are capable of transforming or degrading contaminants to less harmful or immobile forms. However, optimal performance of subsurface bioremediation operations remains a somewhat empirical exercise, a fact that continues to incur significant cost within the DOE complex.



It is clear that moving the technology of bioremediation forward requires improvements in pore-scale imaging techniques suitable for quantifying microbial structure, growth patterns, and biogeochemical pathways three-dimensionally within porous media. BER scientists are developing approaches based on the use of elemental (e.g. Ag, Au) colloidal particles as a contrast agents to physically strain (adsorb to) biomass phases. These researchers are also using labeled antibodies that are attached to features internal to the biofilm. In both cases, the research depends upon the development of high-resolution (nanoscale) visualization technologies of microbial communities in biofilms. The hard and soft X-ray nanotomography instruments that can be developed at NSLS-II will provide these scientists to visualize these films in-situ with unprecedented resolution.

Similarly, as described above, BER scientists have identified microbes that hydrolyze urea, resulting in the co-precipitation of calcium carbonate and Sr.

Thus these microbes are capable of sequestering carbon along with the remediation of radionuclides like  $^{90}\text{Sr}$ . In addition, microorganisms in the family Geobacteraceae have been shown to remediate uranium while harvesting electrical energy via electron transfer to electrodes. In order to take advantage of these characteristics for combined bioenergy production and radionuclide remediation, a systems biology approach to characterization is required. It is necessary to identify mechanisms controlling the expression of key genes related to survival, growth, and activity in subsurface environments and on electrodes.

### **Contaminant uptake, release, and transport in subsurface environments**

The transport of contaminants in the subsurface environment is dependent upon many factors, including the composition and pore size of the sediments and speciation of the contaminants. The reactive transport of contaminants can be modeled in the laboratory environment, but scaling of these geochemical reactions to the field site level presents added challenges.

In order to model these systems, one goal is to link macroscopic measures of contaminant partitioning to the molecular-scale mechanisms that mediate the process. A multi-faceted set of time-dependent, bulk, and spatially-resolved measurements is necessary to develop a mechanistic interpretation of the macroscale data. These include state-of-the-art methods in molecular-scale spectroscopy (e.g. micro-focused EXAFS, FTIR imaging, and solid-state NMR spectroscopy) and micro-scale analyses (e.g. electron microscopy, X-ray fluorescence imaging, and synchrotron X-ray micro- and nano-tomography). Collaborative experimental studies will guide the development of a robust reactive transport model of the coupling between mineral transformation and sorption of Cs, Sr and I in contaminated sediments. The model will be validated based on prior batch and new column scale studies, constrained by the detailed solid-phase analyses, and applied at the field scale using borehole data being collected in association with other projects.

## **Chapter 3: Essential Capabilities**

### Introduction

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NSLS-II will have unparalleled brightness and exceptional stability over the spectral range from infrared light to high-energy X-rays. NSLS-II beamlines will be developed to deliver these beams in support of a broad range of science from fundamental physics to materials science and from basic biology to environmental science. The NSLS-II project is supported by DOE-BES to construct and operate the accelerator systems that will generate beams that will support this broad range of science, as well as an initial suite of six project beamlines aimed at supporting BES science. We anticipate that support for additional beamlines serving BES mission needs will be provided by BES through the mechanism of Major Items of Equipment (MIE) funding. We also anticipate that BES will provide support for transferring some existing research programs from NSLS to NSLS-II through the mechanism of NSLS-II early operations funding.

Beamlines to support synchrotron applications primarily outside the BES mission areas are expected to be developed with support from partner agencies or institutions. Toward that end, NIH recently committed to support the development of four undulator-based beamlines: two for macromolecular crystallography (MX), one for high brilliance small-angle and wide-angle X-ray scattering (XRS) and one for coherent diffraction imaging (CDI), most likely in the hard X-ray regime.

Access to all beamlines at NSLS-II will be open to the entire general user community based on merit as determined by the peer-review beamtime proposal process. As such, BER-supported scientists will have competitive access to all of the instrumentation at the facility, independent of funding sources for construction and/or operation of that instrumentation. For example, although the six project beamlines are primarily intended to serve BES mission needs, their capabilities will also open up new scientific opportunities in other fields and several of them already include biologists and environmental scientists among the Beamline Advisory Team (BAT) members who are helping to specify their scientific missions and technical requirements.

There is a pressing need to establish beamlines at NSLS-II with the capabilities required to address the rich set of opportunities for NSLS-II to contribute to BER science that were identified in Chapter 2. The initial complement of six project BES-funded beamlines and four NIH funded beamlines for which funding is presently committed will not provide all of the capabilities required to support BER science. Several opportunities for BER participation at NSLS-II are identified here that are particularly exciting. It is expected that BER-supported beamlines will be available to all meritorious general users just as BES- and NIH-supported beamlines will be available to meritorious BER scientists.

### BER Participation at NSLS-II

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Opportunities for BER participation at NSLS-II have been considered in light of the unique characteristics of NSLS-II as noted in Chapter 1 and in light of an analysis of BER-related scientific opportunities and needs described in Chapter 2. This analysis also takes into account the NSLS-II beamline development projects mentioned above that are already in progress, emphasizing new capabilities that the currently funded beamlines will not provide. The emphasis is on unique features of the NSLS-II facility that can address exciting and pressing BER science questions. With NSLS-II, unique opportunities in systems biology arise for nanoscale imaging (simultaneous structure and chemistry), high-throughput analyses (feedback to genomics) in such areas as MX, footprinting and XRS, studies of multi-domain complexes, and studies of macromolecular dynamics. There is also a clear demand for multi-scale explorations – molecular, to understand how genes determine biological structure and macromolecular

function; whole cells, to understand how molecular processes are coordinated to perform cell functions; and microbial communities and higher organisms, to understand how cells interact and respond to their environment. In this context, the Biology Village concept is expected to play an important role in facilitating multi-technique approaches for addressing questions of interest.

Application	Source <sup>a</sup>
X-Ray Fluorescence & Absorption Nanotomography	U
Macromolecular Crystallography (MX) with Coordinated Optical Spectroscopy	U
X-Ray Footprinting	DW
Fourier Transform Infrared (FTIR) Microscopy	BM
Soft X-Ray Nanotomography & Spectromicroscopy	U
X-Ray Absorption Spectroscopy (XAS)	3PW
High Throughput X-Ray Scattering (XRS)	3PW

<sup>a</sup> U ≡ undulator; DW ≡ damping wiggler; BM ≡ bending magnet; 3PW ≡ three-pole wiggler

Several prospective beamlines with capabilities that are crucial to BER programs have been identified and are listed in Table 1. For each of these, a one-page description follows that outlines the unique capabilities and necessary beamline technologies. The need for the first four (X-ray Fluorescence and Nanotomography, Macromolecular Crystallography with Coordinated Optical Spectroscopy, X-ray Footprinting, and Fourier-transform Infrared Microscopy) is especially pressing. All of the listed capabilities would have significant impact in advancing the scientific mission of BER and in providing the best support to BER scientists. In addition to the beamlines highlighted here, other beamlines with capabilities that are desirable for BER programs include circular dichroism (CD), transmission X-ray microscopy (TXM), diffraction-enhanced imaging (DEI), and coherent diffraction imaging (CDI).

## X-Ray Fluorescence and Absorption Nanotomography

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### **Unique Capabilities**

X-ray fluorescence and absorption microscopy is invaluable for imaging elemental concentration, distribution, and oxidation state in biological materials. However, imaging at a subcellular resolution has been realized only recently due to advanced X-ray sources, focusing optics, and detectors. XRF microscopy offers unique capabilities stemming from its large penetration length and high chemical sensitivity, allowing thick samples to be imaged in 3-dimensions.

As the caretaker of the nation's genomic database, BER recognizes the full potential of functional genomics is in understanding how genes encode for ion transport. For example, every cell needs to maintain appropriate concentrations of essential nutrient metals while excluding nonessential toxic metals and these genes underpin metal homeostasis. Understanding these mechanisms is the first step in developing plants to feed the increasing global population, to remove metal contaminants from the soil or to exclude toxic metal contaminants from edible tissues, or in developing plants capable of more efficient photosynthesis for production of bioenergy. In the oceans, all nitrogen transformations and many carbon transformations involve metalloenzymes and understanding metal homeostasis is critical in developing models for CO<sub>2</sub> and NO<sub>2</sub> cycling in the atmosphere or in engineering viable sequestration technologies.

While a plethora of new genomics information is now available, a major bottleneck to answering these questions becomes the ability to rapidly visualize -- in three dimensions -- the effects of genetic differences at the nanoscale (organelle) level in the natural cellular environment. The high brightness of NSLS-II provides an unrivaled opportunity to simultaneously acquire anatomic structures and elemental composition of wet or frozen hydrated cells at a spatial resolution of <50 nm. This technique is analogous to multi-modality CT technique in medical imaging, but both the anatomy and chemistry will be acquired at <50 nm resolution, bridging the gap between visible light and electron microscopy.

While an XFM beamline is planned in the initial NSLS-II project suite, its design is optimized for accessing higher energies between 5-25 keV with spatial resolutions >100 nm. BER science is best suited by a beamline accessing lower energies between 2-15 keV with a spatial resolution <50 nm.

### **Experimental Beamline Technologies**

An undulator-based X-ray fluorescence nanotomography (XFN) beamline at NSLS-II will be able to routinely generate images with <50 nm spatial resolution and attogram detection sensitivity. Coupled with the new 384-element Maia detector currently be developed at NSLS, data can be generated hundreds of times faster, enabling 3D imaging of elements at attogram abundance. Furthermore, X-ray absorption spectroscopy can be performed with this nanoscale beam to map the elemental speciation from phosphorus through bromine. Such a beamline could outperform other similar instruments in the U.S. by up to a factor of 50x. 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 in microbial biofilms or at the plant-microbe interface, where microbes foster nutrient cycling in biomass production and environmental remediation. In addition, the "Biology Village" environment of NSLS-II will foster the correlation of XFM imaging with other techniques to co-localize changes in organic composition with trace element composition / speciation.

## Macromolecular Crystallography with Coordinated Optical Spectroscopy

### ***Unique Capabilities***

To perform optical spectroscopy measurements in coordination with macromolecular crystallography (MX) is a powerful way to study biomolecules that contain colored co-factors. As a class, these macromolecules contain transition metals or conjugated organic molecules, and the functions of the complex are typically to catalyze redox reactions or to be carriers for other molecules like O<sub>2</sub>. The opportunities to participate in BER-mission research projects are great. For example, the study of di-iron centers in both the lignin-degrading enzymes at the Great Lakes Bioenergy Research Center by Fox and Phillips, and the lipid desaturase at Brookhaven National Laboratory by J. Shanklin, will both benefit from this experimental method. Similarly, this beamline will significantly aid the search for understanding of lignocellulose-degrading enzymes at BNL by C.J. Liu, and of direct hydrogen production at GLBRC by Hegg and Hausinger, at Univ. of Rochester by J.H. Wu, and at U. Mass Amherst by D. Lovley. All of these are BER-funded projects except for Shanklin, who is funded by BES.

X-ray crystallography can provide a unique opportunity to study the structures of macromolecules while monitoring the state of redox or liganding cofactors. In particular, an X-ray diffractometer can be equipped with a micro-beam spectrometer that allows optical spectroscopic studies on the same volume of a crystal that is illuminated by the X-ray beam. Several spectroscopic methods can be employed: UV-Visible absorption, UV-Vis fluorescence and fluorescence excitation, Raman, resonance Raman, and InfraRed. In principle, the light (and X-ray) beam could be as small as a micrometer or so. In practice, beams of light and X-rays might be made to intersect reliably in the tens-of-micrometer range. This is completely adequate, both to create specimens this size and to get sufficient material in the light beam to perform spectroscopic measurements.

The final feature of this experimental mode is that the redox state of the specimen may be controlled by the X-ray beam itself: absorption of X-rays by the crystal creates photo-electrons, which **will** alter the redox state of the cofactor. To observe spectral and structural changes during this process is surprisingly useful in teasing out a redox enzyme's function.

Therefore, the communities of investigators that could be served will require access to an experimental station at NSLS-II that will allow coordinated measurement of spectroscopic and diffraction data on each specimen. Because high-speed measurements to capture transient species could be useful, these experimenters should have access to the very bright source of an NSLS-II undulator beamline. This beamline would participate in the traditional BER cooperation with NIH and other agencies to provide synchrotron access for the national structural biology community.

### ***Experimental Beamline Technologies***

To create a fully-functioning spectroscopy lab, which also is capable of performing very difficult crystallographic measurements, an NSLS-II undulator beamline would be excellent. It would provide tunable X-rays with beam sizes in the range 5 – 100 μm, and it would be especially useful either for very small specimens or for high-speed measurement in performing spectroscopic measurements concurrently with diffraction. This would be a facility unique in the US. The emphasis would be on stable sample beams of 25μm or smaller, and robust and reliable operation. Also important is to switch between spectroscopic modes while a single specimen is mounted on the X-ray diffractometer. This undulator station will also serve all other BER mission-motivated crystallographic projects.

## X-Ray Footprinting of Proteins and Nucleic Acids

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### **Unique Capabilities**

Over the last 10 years, synchrotron-based X-ray footprinting using a white beam has been successfully applied to study the structure and dynamics of a variety of biological systems. The technique, pioneered at NSLS, relies on the generation of hydroxyl radicals through the interaction of X-ray beams with water (radiolysis). The radicals are generated isotropically in the solution phase, and they covalently modify solvent-accessible bases on nucleic acids or side chains of proteins, providing a sensitive probe of local structure. The structural data are read out using mass spectrometry analysis for proteins or high-resolution gel electrophoresis of nucleic acids. The technique is unique in its ability to provide high-resolution solution-phase information on local structure of macromolecules, and it can be applied to assess the dynamics of evolving systems in real time on microsecond or longer timescales.

With the growing interest in biology to study the structure and dynamics of large biomolecular assemblies, membrane proteins, and *in vivo* systems, footprinting is becoming increasingly important. The technology has several powerful features. First, footprinting approaches are not limited by the size of the molecule, and are quite suitable for examining structures of complex systems. Second, footprinting data can be collected using only nanograms of sample under various solution conditions, thus allowing examination of systems that are available only in small amounts and in near physiological states. Third, footprinting approaches have been demonstrated to be applicable to examination of molecular structure in cells, providing entirely new avenues for such studies.

Of great significance is that footprinting has now been shown to provide a probe of water molecules within proteins and can probe the dynamics of water exchange in ion transport. These studies clearly show the importance of this technique to analyze structure and dynamics of membrane proteins and large macromolecular complexes for BER- and BES-mission research projects. For example, studies attempting to determine solvent accessible domain and substrate binding pockets of hydroxylase, and to determine the conformation dynamics of native/active states of Zinc transporter and the mechanism of  $Zn^{2+} / H^{+}$  transport across the membrane, will both be revolutionized by X-ray footprinting. This approach will also provide significant breakthroughs in studies to determine the higher order structure and interaction surfaces of the enzyme complexes in lignin biosynthesis and cellulose degradation pathways. The X-ray footprinting method will provide a unique quantitative tool to study structure-function relationships for many bioenergy and environmental related research projects where high resolution structures are not available for large complexes and membrane proteins and where dynamics of the evolving system need to be understood to understand mechanism.

### **Experimental Beamline Technologies**

To realize the full potential for the technology, increased X-ray flux-density on the sample is required to reduce the overall exposure time from the current level of ~1 ms. Such increased flux-density could reduce the quenching effect of hydroxyl radical scavengers and will permit microsecond exposure times for unique static and time-resolved studies. NSLS-II, which provides unique Damping Wiggler technologies, would be a revolutionary source for these footprinting technologies. An NSLS-II damping wiggler beamline would provide 100-fold increases in flux density and exposure times in the 10-microsecond range permitting currently impossible studies of dynamically evolving membrane proteins and large macromolecular complexes both *in vitro* and *in cells*. X-ray footprinting data is also highly complementary to other synchrotron-based structural biology techniques, such as crystallography and SAXS, making it a natural member of a multi-disciplinary "Biology Village."

## *In Vivo* Fourier Transform Infrared Imaging

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### ***Unique Capabilities***

Fourier Transform InfraRed (FTIR) microscopy is a spectroscopic imaging technique that probes the organic composition of biological cells and tissues on a microscopic scale without the need for stains or labels. Compared to a conventional laboratory instrument, the high brightness of synchrotron infrared light dramatically improves the spatial resolution such that individual cells can be probed with diffraction-limited spatial resolution (i.e. 2 – 10  $\mu\text{m}$ ). Moreover, since infrared light is non-ionizing, cells can be studied in their natural environment as a function of time. For example, recently synchrotron FTIR microscopy has been used to characterize bacterial activity in biofilms, metabolite formation in algae, and protein structure in alfalfa.

Currently, FTIR microscopy beamlines exist on over 25 synchrotrons worldwide including four beamlines at NSLS. This large number of beamlines illustrates the high demand from the user community; however, the uniqueness of NSLS-II has the potential to revolutionize the technique worldwide. First, the high brightness of NSLS-II will translate into an improvement in spatial resolution to  $< 1 \mu\text{m}$  when image deconvolution routines are implemented. Second, the ultra-low noise electron beam will translate into an improvement in signal-to-noise by a factor of 10 – 100, dramatically improving the detection sensitivity of the technique. Third, the unique dipole chamber design will permit the extraction of wide swath of beam to illuminate an infrared pixel array detector, which translates into the unique ability to image a large region of a sample quickly, without the need for raster-scanning the sample through the beam.

These unique characteristics of NSLS-II will move BER science in exciting new directions such as cellulose degradation by bacteria, where the reaction location, rate, and resulting chemical intermediates can be determined in an intact lignocellulose-microbe system at a subcellular spatial resolution; microbial fixation of  $\text{CO}_2$  into calcium carbonate, where the formation of the mineral can be tracked by unique FTIR spectral signatures and correlated with the composition and distribution of microbial communities; and microbial reduction and sequestration of uranium, where FTIR imaging can be used to characterize the reaction products – specifically the spatial distribution and composition of the uranium precipitates. Importantly, these images can be combined with XFM and micro-XANES in order to correlate the microbe / mineral composition to the distribution and oxidation state of uranium, respectively.

### ***Experimental Beamline Technologies***

Existing synchrotron infrared microscopes are limited by slow data collection rates, millimolar detection sensitivity, and diffraction-limited spatial resolution. The high brightness, low noise, and distinctive dipole magnets of NSLS-II have the potential to revolutionize infrared spectroscopic imaging. Coupled to an environmental chamber such as those designed by BER investigators, an FTIR imaging beamline at NSLS-II can be used for *in vivo* studies on time scales from microseconds to days with micromolar detection sensitivity and sub-micron spatial resolution. Moreover, the “Biology Village” environment of NSLS-II will foster the correlation of FTIR and XFM imaging (among others) to co-localize changes in organic composition with trace element composition / speciation, which will permit BER-relevant studies such as microbe-surface interactions, bacterial activity in biofilms, cell exposure to toxins, nutrient cycling in plant-microbe interactions, and biomass catalytic breakdown.

## **Soft X-Ray Spectromicroscopy and Nanotomography**

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### ***Unique Capabilities***

Soft X-ray microscopes occupy a unique position among the many imaging techniques available. Chemical contrast at spatial resolutions of 30 nm can be obtained from unsectioned cells with thicknesses of 5–50  $\mu\text{m}$ , without labeling. By acquiring image sequences at finely spaced energies across an X-ray absorption edge, information is obtained on the chemical binding states of the element; for example, at the carbon edge one can distinguish between many different organic functional groups when present at 1% local concentration. By acquiring tilt series of images or lensless diffraction patterns, X-ray microscopy obtains quantitative, 3D views of submicron particles. In cells and biofilms, protein, lipid, and polysaccharide can be quantitatively mapped.

Cell imaging is important to environmental and energy science as well: metals biomineralized by bacteria in mine drainage systems can be imaged and analyzed for oxidation state. The nature of organic species associated with clay minerals is significant in several processes, from hydrocarbon recovery in oil sands to contaminated soil remediation and water treatment; X-ray microscopy can identify these absorbates. Recent applications of synchrotron X-ray spectromicroscopy also include studies of spore walls and pollen grains, where reference spectra of sporopollenin and cellulose are applied to interpret ultrastructures in fossil and modern grains. This last application has obvious implications for in-situ imaging of plant biomass conversion reactions. In-situ imaging of swelling polymer microgels in aqueous solution has been demonstrated already, and this has direct relevance to feedstock pretreatment and hydrolysis studies.

Soft X-ray microscopes, which are universally oversubscribed at synchrotrons worldwide, operate over the energy range from 280 eV to about 3 keV. At the lower end of this range lies the carbon K edge, where near-edge absorption resonances can be used to map the distribution of organic functional groups. While chemical imaging opportunities exist at many low-Z elemental edges, photon energies just below the oxygen edge provide maximum contrast for transmission imaging of smaller whole cells. Above 1500 eV, phase contrast comes into play, offering greater x-ray penetration for imaging thicker samples. In full-field transmission x-ray microscopes (TXM), a Fresnel zone plate is used to produce a magnified image on a 2D detector. Because the image pixels are acquired in parallel, these microscopes acquire data rapidly even at bending magnets; this has traditionally made TXM preferable for nanotomography, where hundreds of images are required; however, the high brightness of NSLS-II makes nanotomography feasible in a scanning microscope geometry, with tenfold lower dose to the specimen and thus higher practically achievable resolution. In scanning transmission x-ray microscopes (STXM), a Fresnel zone plate produces a point focus through which the specimen is scanned. For finest focus and energy resolution, these microscopes are best operated from high brightness beamlines.

### ***Experimental Beamline Technologies***

NSLS-II has the capability to revolutionize X-ray microscopy due to its high brightness and extremely stable ring. Current constraints of electron beam noise and available zone plate optics limit spot size to 25 nm, but 10 nm will be achieved with upcoming advances. Development is needed in the area of cryo methods, essential for studying life science specimens. Decades of development of cryo preparation for electron microscopy have arrived at good solutions for quantitative, low-dose imaging. X-ray microscopy extends the challenge to include the necessity for scanning and the increased sample thicknesses. Cryogenic robotics for sample changing is essential for high throughput, as are advances in computing and data analysis.

## Advanced X-Ray Absorption Spectroscopy

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### ***Unique Capabilities***

A common theme in biofuel production, carbon biosequestration, and contaminant biogeochemistry is the need for understanding the structure and function of enzymes and proteins intimately involved in the processes. About one-third of all proteins require a metal atom for their specific structure and function; these metalloproteins function in a variety of processes including those involved in biological energy production and bioremediation. For this reason, X-ray absorption spectroscopy (XAS) has long been of critical importance to environmental and biochemical research, particularly studies related to understanding and manipulating hydrogen production in microbes. These studies are challenging because they generally require collection of data on highly dilute samples or *in situ* in soils. Environmental systems with metals of interest include microbes, soil minerals, nanoparticles, and solutions. A complex exchange of chemicals, nutrients, electrons, and carbon among these components, has a strong influence on biomass productivity, carbon cycling, and contaminant fate and transport. An important subset of these processes is redox cycling; electron transfer processes involve biological and inorganic components, and control many aspects of metal speciation and carbon recycling.

XAS has typically been used as a static probe of metalloprotein structure; understanding the evolving dynamics of metalloprotein systems is a critically important new area. The application of continuous-flow mixing technology can provide the opportunity to extend XAS measurements into the microsecond time-scale, potentially revolutionizing the study of biochemical and bioinorganic systems and providing a real-time probe of the catalytic mechanisms mediated by metal atoms. Projects funded by BER, including the MURMoT project for microbial uranium reduction monitoring which anticipates observation of uranium reactions in real-time, are already poised to take advantage of this capability. Although biological XAS has primarily focused on the transition metal K absorption edges (6-12 keV), there is need to expand to other elements. Higher energy XAS includes K edges of heavy elements such as Cd, Se, Pd, Ag, Mo and Sb, and offers improved sample penetration. Lower energy XAS shows great potential for biologically-important lighter elements (Ca, S, P, Mg), advantageous use of heavier element L and M absorption edges, and also combined studies of redox reactions and speciation of both light and heavy elements in a single analysis. XAS complemented by other imaging technologies also provides critical chemical information and can probe target elements in their natural state in a variety of laboratory and field-collected samples.

### ***Experimental beamline technologies***

To revolutionize the Nation's biological and environmental research programs, expanded energy ranges (novel elemental analysis), brighter beams (imaging and time-resolved studies), and increased flux (dilute samples and *in situ* studies) are all critically needed. A number of unique features of NSLS-II can provide technological solutions to many of these challenges. In particular, the 3PW sources are well suited for biological and environmental XAS with spatial resolution from sub-um to mm scales. They will provide total flux and energy comparable to NSLS bending magnets, permitting transition strategies for existing BER XAS programs, but with an order of magnitude higher brightness to enhance microbeam XAS capabilities.

## High-Throughput Solution X-Ray Scattering

### ***Expected Capabilities***

X-ray scattering (XRS) provides direct structural information on non-crystalline structures that cannot be solved by high-resolution structural characterization methods such as crystallography and NMR. These measurements can be performed on structures (e.g. proteins in solutions) that are actively participating in biological and environmental processes that are relevant to BER's mission. For instance, for bioenergy applications, X-ray scattering is being used to elucidate the cooperative interaction between individual enzymes in bacterial cellulosomes that are responsible for degradation of lignocellulosic biomass and to monitor the structure of artificially constructed, high-efficiency cellulosomes as building blocks are added to the scaffold. The structure of the biomass can also be followed during degradation in the presence of a cocktail of these designer enzymes via a combinatorial approach. XRS has also been used to aid the effort of genomics by providing low-resolution characterization on large numbers of protein products. In the field of environmental remediation, XRS has been used to study contaminant adsorption at mineral-water interfaces.

XRS from biomolecular solutions has been attracting more and more researchers in recent years because of its ability to follow biomolecular structures in near native environment. Low-resolution scattering data can be combined with high-resolution but rigid or partial structures, such as those obtained from crystallography, NMR and EM, to provide a more complete understanding of the structure and function of the biomolecules. The simplicity of using low resolution structural information (e.g. molecular shape) obtained from simple measurements to answer specific question (e.g. activity of a ligand) also makes XRS ideal for combinatorial-type research and the genomics effort. In these examples, a large number of proteins need to be each characterized under a variety of chemical conditions, e.g. at different pH values or in the presence of ligands from a chemical library.

Clearly the unprecedentedly high source brightness at NSLS-II presents exciting scientific opportunities. It will provide new capabilities to perform time-resolved scattering measurements from biomolecules in solution for kinetic studies that are essential to understand the functions of biological molecules and to study in grazing incident geometry two-dimensional membrane structures that have extremely small sample volumes. Recognizing these opportunities, NIH has already committed to build an undulator-based beamline that is optimized for these brightness-limited measurements. While static solution scattering measurements can also be performed on this beamline, this beamline will be designed for flexibility to accommodate frontier measurements under various beamline configurations rather than rapid data collection from large numbers of samples using similar scattering configuration. It is also unlikely that this single beamline will be able to support all biological and environmental science research requiring solution scattering that will take place at NSLS-II. Therefore it is appropriate and necessary to have a separate high throughput solution scattering beamline dedicated to combinatorial and genomics research. The limited beam time will be more efficiently utilized through automation of data collection and analysis. Scattering data at small angles and wide angles will be recorded simultaneously to minimize the need for instrument reconfiguration. Sample characterization using other techniques, such as optical spectroscopy, can also occur in parallel with the X-ray measurement.

### ***Experimental Stations***

This dedicated high-throughput beamline will use a 3PW source to carry out static solution scattering measurements using high throughput instrumentation and software that are under development at NSLS. Focusing optics designed to match the characteristics of the 3PW source and a high band-pass multilayer monochromator will provide the flux necessary for high throughput measurements.