Molecular Interactions, Protein Complexes, and Structural Biology

The ENIGMA Project: Mapping Protein Assemblies and Modifications by Cellular Deconstruction and Mass Spectrometry in the Hyperthermophiles *Sulfolobus solfataricus*, *Pyrococcus furiosus*, and *Halobacterium NRC-1*

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Project Goals: As part of the integrated ENIGMA project our goals are to develop generalized methodologies for identifying and isolating macromolecular assemblies and modified proteins from native biomass. We aim to identify and characterize both stable and transient macromolecular assemblies within the cell, the cell membrane, and outside of the cell using a rational cellular deconstruction approach. Proteins are identified by MS/MS from our cellular deconstruction fractions that have inherent cellular locality, assembly mass, protein modification, and potential partner protein information associated with their identification. Our proteome-wide approach to identify macromolecular assemblies and protein modifications will ultimately lead to identification of functional modules suitable for transfer between microbes. We are developing generally applicable molecular and biophysical technologies for GTL to ultimately apply functional modules to confer specific metabolic capabilities to microbes and address DOE mission goals.

Dynamic protein-protein interactions are fundamental to most biological processes and essential for maintaining homeostasis within all living organisms. These interactions create dynamic and diverse functional networks essential to biological processes. Thus, a thorough understanding of these networks will be critical to engineering biological processes for DOE missions. The project was conceived, in part, as a response to the DOE GTL initiative to develop technologies to map the proteomes of model organisms. In this project we are exploiting unique characteristics of members of extremophilic Archaea to identify, isolate, and characterize multi-protein molecular machines. We have teamed expertise in mass spectrometry, systems biology, structural biology, biochemistry, and molecular biology to approach the challenges of mapping relatively simple proteomes.

As part of the ENIGMA project, we have developed methods for whole cell deconstruction of microbes that separates intact protein complexes under native conditions. The cellular deconstruction primarily fractionates the organism into four major classes: 1) membrane, 2) large mass (>800 kDa), 3) small mass (<800 kDa), and 4) extracellular. The final cellular partitions were resolved on SDS-PAGE and excised for high throughput MS/MS protein identification at the Scripps Center for Mass Spectrometry. We have achieved over 50 percent coverage of the predicted proteome and all identifications have inherent subcellular locality and co-fractionation information associated with them: 1) proteins identified in sedimenting fractions are by definition members of large mass complexes, 2) isolated membrane vesicles allow identification of membrane associated proteins, and 3) extracellular proteins contain extracellular membrane-bound and secreted proteins groups. We are refining analysis tools to identify co-variations of proteins across the fractionation scheme to build a system-wide protein interaction network. Ultimately, we aim to identify metabolic modules suitable to transfer specific metabolic processes between microbes to address specific DOE mission challenges while developing generally applicable molecular and biophysical technologies for GTL.

High-Pressure Cryocooling of Protein Crystals: Applications to Understanding Pressure Effects on Proteins

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Project Goals: Understand effects of pressure on proteins.

A novel high-pressure cryocooling technique for preparation biological samples for x-ray analysis has been developed [1-3]. The method, high-pressure cryocooling, involves cooling samples to cryogenic temperatures (~100 K) in high-pressure Helium gas (up to 200 MPa). It bears both similarities and differences to high-pressure cooling methods that have been used to prepare samples for electron microscopy, and has been especially useful for cryocooling of macromolecular crystals for x-ray diffraction. Many different kinds of macromolecular crystals have been successfully high-pressure cryocooled and excellent crystal diffraction has been obtained with little or no penetrating cryoprotectants.

This new method has great potential for understanding pressure effects on proteins. As an example, high pressure cryocooling has been used to understand the structural basis for why the emission spectrum of the protein, Citrine, is pressure dependent [4,5]. The deformation of the Citrine chromophore is actuated by the differential motion of two clusters of atoms that compose the β-barrel scaffold of the molecule, resulting in a slight bending of the β-barrel. The high-pressure structures also reveal a perturbation of the hydrogen bonding network that stabilizes the excited state of the Citrine chromophore. The perturbation of this network is implicated in the reduction of fluorescence intensity of Citrine. The blue-shift of the Citrine fluorescence spectrum resulting from the bending of the β-barrel provides structural insight into the transient blue-shifting of isolated yellow fluorescent protein molecules under ambient conditions and suggests mechanisms to alter the time-dependent behavior of Citrine under ambient conditions. Finally, the Citrine example serves as a model for the way in which global pressure-induced structural perturbations affect the activity of proteins, as well as a model for how these perturbations may be studied.

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and 3Dept. of Biochemistry and Molecular Biophysics, Source, Brookhaven National Laboratory, Upton, N.Y.; time- and spatial-resolution that cannot be achieved today. spectroscopy (XAS), and extend them to new regimes in small-angle x-ray scattering (SAXS), and x-ray absorption methods, such as macromolecular crystallography (MX), But importantly, NSLS-II will also take widely utilized ing, that are currently in their infancy or do not exist today. enable characterization techniques, such as nanoscale imag- tion, multi-technique integration, and cross-disciplinary these communities to see increased interaction, collabora- Environmental Sciences workshops was the desire within NSLS-II. An overarching conclusion from the Life and 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.

The 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.

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.

At the NSLS today, biological and environmental sciences users represent approximately 60% of the user community and more than 650 of the facility’s annual publications. 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, collabora- tion, 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 "Biol-
ogy 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.

In this poster, we will describe how the unique characteristics of NSLS-II can address scientific problems relevant to BER scientists by presenting a series of applications to bioenergy, carbon cycling and sequestration, and contaminant transport and cleanup in the environment. We will emphasize the wide range of techniques that will permit multiscale exploration: at the molecular level, to understand how genes determine biological structure and function; at the cellular level, to understand how molecular processes are coordinated to execute cell function; and at the level of microbial communities and higher organisms to understand how cells interact and respond to their environment. Finally, we are eager to solicit new applications of synchrotron science to research problems of interest to BER scientists.