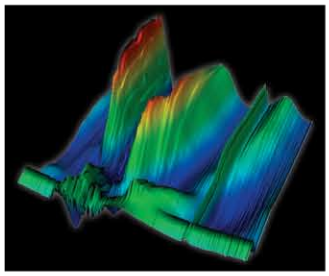
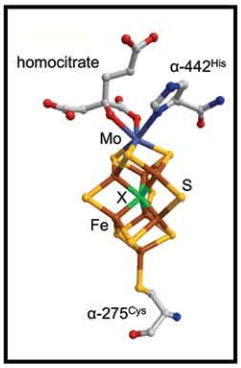
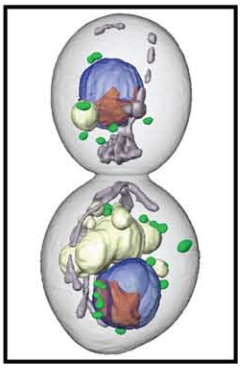
DOE User Facilities

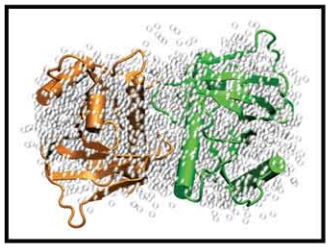
Advanced Technologies for Biology

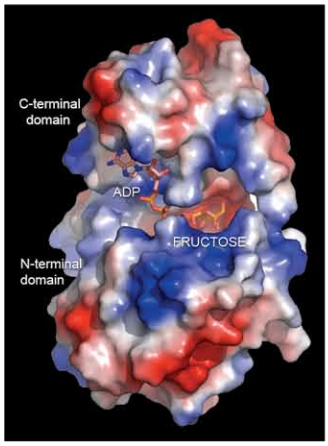
Synchrotron and Neutron Beam Facilities Accelerating Biological Research

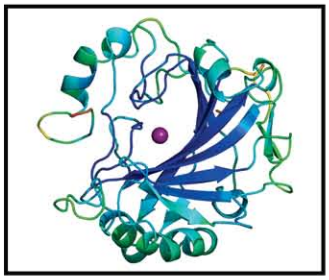


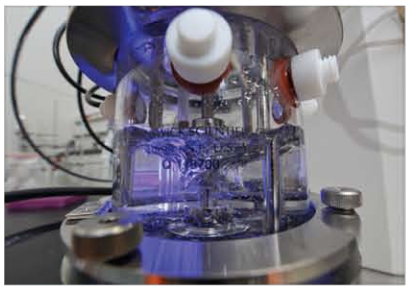


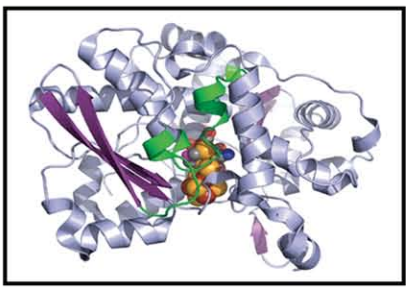


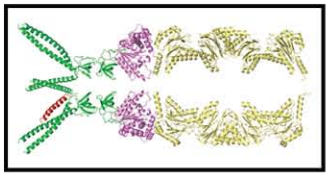












U.S. DEPARTMENT OF ENERGY | Office of Science

Office of Biological and Environmental Research

About the Cover

Image credits from top to bottom, left to right. Three-dimensional diagram of *Desulfovibrio vulgaris* cell chemistry courtesy of Lawrence Berkeley National Laboratory (LBNL). Robot in action courtesy of SLAC National Accelerator Laboratory. MoFe nitrogenase structure courtesy of LBNL. Tomographic image of *Saccharomyces cerevisiae* cell courtesy of LBNL. PYR1 protein structure courtesy of LBNL. ROK fructokinase structure from *Bacillus subtilis* courtesy of Argonne National Laboratory. Protein Crystallography Station courtesy of Los Alamos National Laboratory (LANL). Ribbon diagram of human carbonic anhydrase II courtesy of LANL. Ribbon diagram of tuberculosis proteasome courtesy of Brookhaven National Laboratory. Bio-deuteration system courtesy of Oak Ridge National Laboratory. Ribbon diagram of [FeFe]-hydrogenase courtesy of SLAC National Accelerator Laboratory. Fourier transform infrared microscopy instrumentation at the Advanced Light Source courtesy of LBNL.

Advanced Technologies for Biology

DOE Synchrotron and Neutron Beam Facilities Accelerating Biological Research

genomicscience.energy.gov/userfacilities/structuralbio.html

Synchrotron light sources and neutron facilities at the Department of Energy's (DOE) national laboratories enable understanding of the structure of matter down to the atomic or molecular level using approaches not possible with laboratory instrumentation. Synchrotron facilities produce intense beams of X-rays and other wavelengths in the infrared to terahertz region, and neutrons are produced from particle accelerators or reactors. The beams are directed into experimental stations housing instruments configured for specific biological investigations.

This infrastructure provides user access to beamlines and instrumentation for high-resolution studies of biological organisms and molecules for all areas of research in the life sciences. Users are chosen through a peer-reviewed proposal process managed by each facility. Capabilities of and contact information for each station are described in the following pages. To find out more about what each experimental program offers, contact the facilities directly.

This activity is supported by DOE's Office of Biological and Environmental Research within the Office of Science and is closely coordinated with other federal agencies and private organizations.

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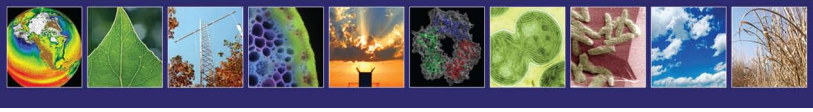
Office of Biological and Environmental Research



U.S. DEPARTMENT OF ENERGY | Office of Science

science.energy.gov/ber/

Structural Biology Center (SBC)

Location: *Advanced Photon Source, Argonne National Laboratory*
Website: *www.sbc.anl.gov*
Principal Investigator: *Andrzej Joachimiak, andrzejj@anl.gov, 630-252-3926*
User Contact: *Stephan Ginell, ginell@anl.gov, 630-252-3972*

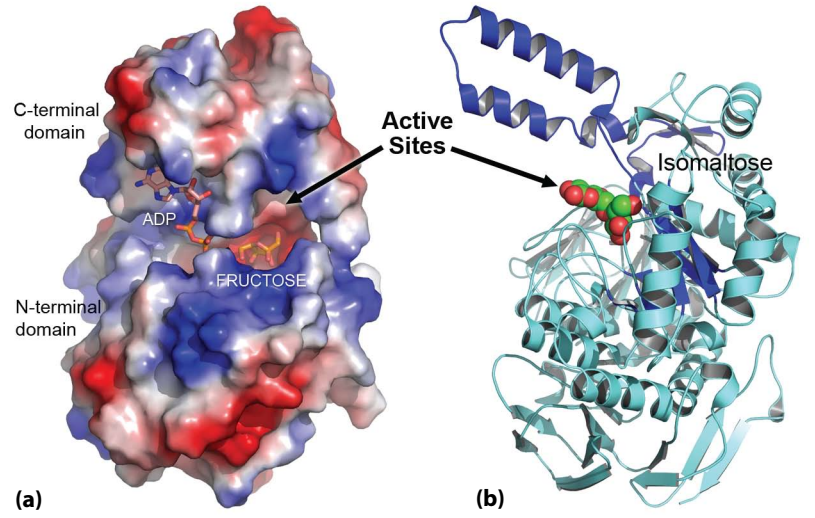
SBC is a major protein crystallography research facility that enables the atomic-scale study of macromolecular systems using extremely small (micron-size) crystal samples. SBC's two experimental stations—the insertion-device beamline and the bending-magnet beamline—are among the most powerful and focused X-ray sources available for structural biology. Output is enhanced by on-axis sample viewing optics; easy access to minibeams (5, 10, and 20 µm) and variable beam sizes (25 to 250 µm); integration of computing and data-storage resources to accelerate data analysis and archiving; near real-time data interpretation, optimization of experimental parameters, and structure solution; and full integration of synchrotron hardware, detectors, crystal mounting robot, beamline software, and crystallographic software packages. These capabilities provide not just diffraction data, but also an interpretable electron density map and a macromolecular structure.

SBC's beamlines can be used for a wide range of crystallographic experiments involving:

- Crystals of macromolecular assemblies with very large unit cells
- Multi- or single-wavelength anomalous diffraction (MAD/SAD) phasing
- Crystals of membrane proteins
- Small, weakly diffracting crystals
- Ultra high-resolution crystallography
- Cryo-crystallography

SBC User Highlight

Understanding Enzymes That Process Sugars and Carbohydrates. In two separate studies, researchers used high-resolution synchrotron protein crystallography to determine the crystal structures of ROK (bacterial **R**epressors, uncharacterized **O**pen reading frames, and sugar **K**inases) fructokinase from *Bacillus subtilis* and a recombinant α-glucosidase from the human gut bacterium *Ruminococcus obeum*. The results provided new information about how enzymes bind, recognize, and process carbohydrate substrates and how variations in enzyme structure impact enzyme function. These findings are expected to improve the conversion of biomass to fuels by using structural information to optimize enzymes for bioprocessing. [[1]Nocek, B., et al. 2011. "Structural Studies of ROK Fructokinase YdhR from *Bacillus subtilis*: Insights into Substrate Binding and Fructose Specificity," *Journal of Molecular Biology* **406**, 325–42. [2]Tan, K., et al. 2010. "Novel α-Glucosidase from Human Gut Microbiome: Substrate Specificities and Their Switch," *The FASEB Journal* **24**, 3939–49.]



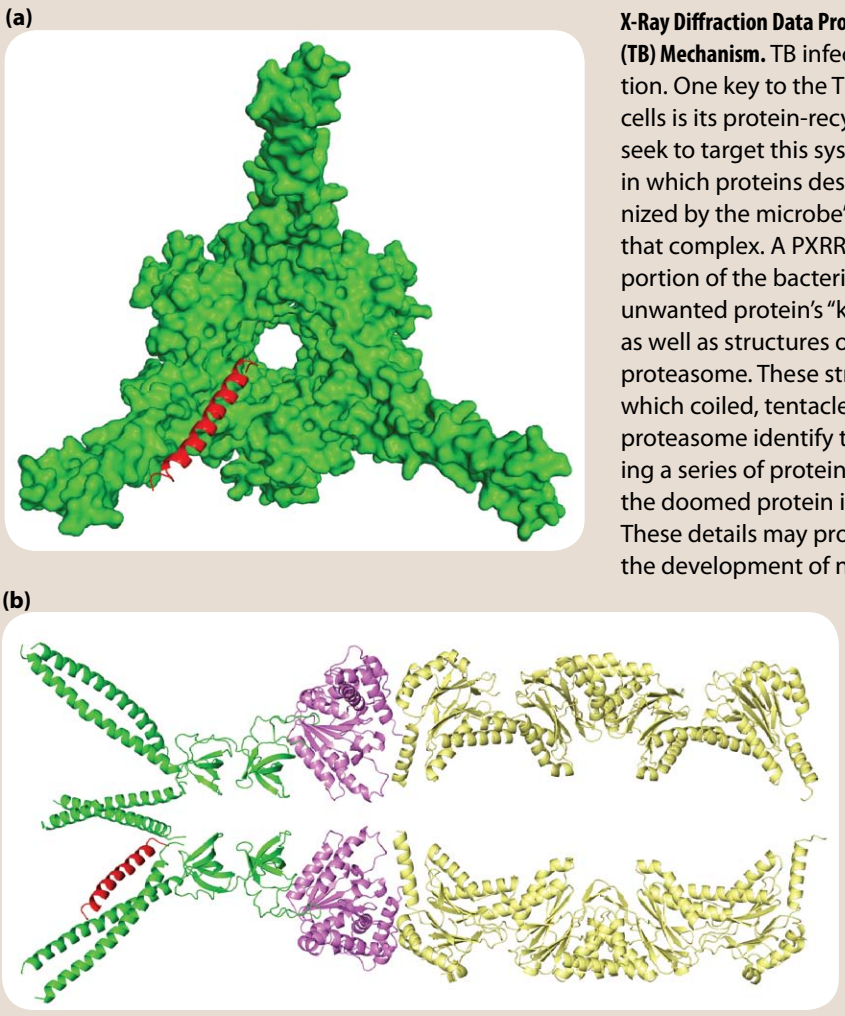
(a) *Fructose and ADP bound to ROK fructokinase from* B. subtilis *(see reference 1).*
(b) *Catalytic domain of α-glucosidase with bound isomaltose from* R. obeum *(see reference 2). [Image credits: Argonne National Laboratory]*

Macromolecular Crystallography Research Resource (PXRR)

Location: *National Synchrotron Light Source, Brookhaven National Laboratory*
Website: *www.px.nsls.bnl.gov*
Principal Investigator: *Robert Sweet, sweet@bnl.gov, 631-344-3401*

PXRR provides facilities and support for macromolecular structure determination by synchrotron X-ray diffraction. Five PXRR beamlines, two of which are high-brightness undulators, allow for highly efficient structure determination by every available technique. Complementary spectroscopic methods, including optical absorption spectroscopy and Raman spectroscopy, enable simultaneous measurements of the same sample under nearly identical experimental conditions. PXRR also provides a popular mail-in crystallography program, builds new facilities, advances automation, and develops remote data-collection capabilities.

PXRR User Highlight



X-Ray Diffraction Data Provide First Insights into Key Tuberculosis (TB) Mechanism. TB infects a third of the world's population. One key to the TB bacterium's survival in human cells is its protein-recycling mechanism. Researchers seek to target this system by understanding the way in which proteins destined for degradation are recognized by the microbe's proteasome before they enter that complex. A PXRR structural study revealed the portion of the bacterial proteasome that identifies the unwanted protein's "kiss of death" marker sequence, as well as structures of this sequence as it binds to the proteasome. These structures suggest a mechanism by which coiled, tentacle-like arms protruding from the proteasome identify the death-sentence label, causing a series of protein-folding maneuvers that pull the doomed protein into the degradation chamber. These details may provide highly specific targets for the development of new anti-TB therapies. [Wang, T., K. H. Darwin, and H. Li. 2010. "Binding-Induced Folding of Prokaryotic Ubiquitin-Like Protein on the *Mycobacterium* Proteasomal ATPase Targets Substrates for Degradation," *Nature Structural and Molecular Biology* **17**, 1352–57.]

Computer-Generated Images of TB Protein-Recycling Mechanism* (a) *Top View and* (b) *Side View. *The images show how the "kiss of death" protein marker sequence (red) is recognized by and binds to one of three long, tentacle-like structures of an enzyme (green) that sits at the entrance to the TB bacterium's protein-degradation chamber, or proteasome core particle (shown to the right of the green region in the side view). The enzyme will unfold the marked protein (not shown here) and feed it into the proteasome core for degradation. [Image credit: Brookhaven National Laboratory]*

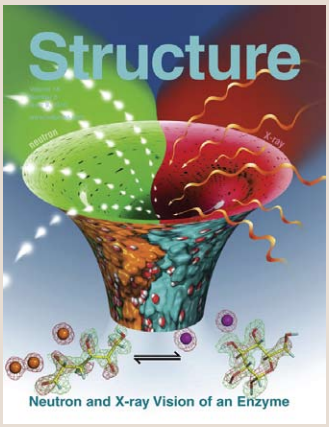
Protein Crystallography Station (PCS)

Location: *Los Alamos Neutron Science Center, Los Alamos National Laboratory*
Website: *lansce.lanl.gov*
Principal Investigator: *Paul Langan, langan_paul@lanl.gov, 505-665-8125*
User Contact: *Zoë Fisher, zfisher@lanl.gov, 505-665-4105*

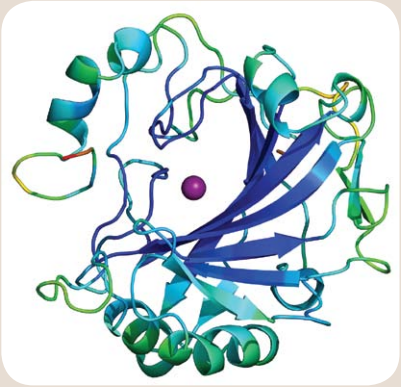
PCS is a high-performance neutron beamline that forms the core of a capability for investigating the structure and dynamics of proteins, biological polymers, and membranes. Neutron diffraction is a powerful technique for locating hydrogen atoms, which can be hard to detect using X-rays, and therefore can provide unique information about how biological macromolecules function and interact with each other and smaller molecules. PCS users have access to neutron beam time, deuteration facilities, technologies for studying protein expression and substrate synthesis with stable isotopes, a purification and crystallization laboratory, and software and support for data reduction and structure analysis. A HomeFlux X-ray system was recently acquired to allow users to collect X-ray data from the same samples used for neutron diffraction. The PCS beamline exploits the pulsed nature of spallation neutrons and a large electronic detector to collect wavelength-resolved Laue patterns using time-of-flight techniques. Data are collected efficiently and with good signal to noise using all available neutrons [with a wavelength range of ~0.7 to 7.0 angstroms (Å)] in the pulsed white beam.

PCS User Highlights

New Insights into D-Xylose Isomerase (XI). XI is an important enzyme because it can convert sugars that resist bioconversion to fuel into those readily fermented by, for example, yeasts. Through neutron diffraction experiments, researchers were able to map the positioning of individual hydrogen atoms as XI moves them from one carbon to another on a sugar molecule. They were able to model how specific amino acids in the XI structure are involved in proton movement. Results may enable new approaches for modifying the enzyme to improve its performance for biofuel and other applications. This research was featured on the June 9, 2010, cover of *Structure*. [Image reprinted with permission from Elsevier: Kovalevsky, A. Y., et al. 2010. "Metal Ion Roles and the Movement of Hydrogen During Reaction Catalyzed by D-Xylose Isomerase: A Joint X-Ray and Neutron Diffraction Study," *Structure* **18**, 688–99.]



Neutron Crystallography Reveals How Carbonic Anhydrases (CAs) Work. CAs are a family of enzymes that play an essential role in the metabolism of carbon dioxide by converting it into a carbonate ion and a proton. Because they are very stable and inexpensive, CAs could be used in significant large-scale applications such as carbon sequestration processes and biofuel production. However, little is known about the arrangement of the active site of CAs while they carry out their function, a gap that has impeded design of optimized CAs for these applications. Neutron crystallography experiments to determine the structure of human carbonic anhydrase II have revealed the orientation of amino acids around the zinc ion in the active site, as well as the unexpected presence of a water molecule bound to the metal ion. This structural information has enabled development of a mechanism to explain the proton transfer process and is being used to re-engineer the enzyme to be pH insensitive and thermally stable for carbon sequestration or biodiesel production. [Fisher, S. Z., et al. 2010. "Neutron Structure of Human Carbonic Anhydrase II: Implications for Proton Transfer," *Biochemistry* **49**, 415–21.]



Ribbon Diagram of Human Carbonic Anhydrase II. *The zinc in the active site is shown as a magenta sphere. The protein's coloring is based on temperature factors, with blue indicating the least amount of movement and red as the most flexible areas of the protein. [Image credit: Los Alamos National Laboratory]*

Structural Molecular Biology (SMB) Center

Location: *Stanford Synchrotron Radiation Lightsource, SLAC National Accelerator Laboratory*

Websites: *www-ssrl.slac.stanford.edu/science/smbgroup.html*

Principal Investigators: *Britt Hedman, hedman@slac.stanford.edu, 650-926-3052; Keith Hodgson, hodgson@slac.stanford.edu, 650-926-3153*

User Contact: *Lisa Dunn, lisa@slac.stanford.edu, 650-926-2087*

The SMB Center's core areas of technological research and development and scientific focus are macromolecular crystallography (MC), X-ray absorption spectroscopy (XAS), and small-angle X-ray scattering and diffraction (SAXS).

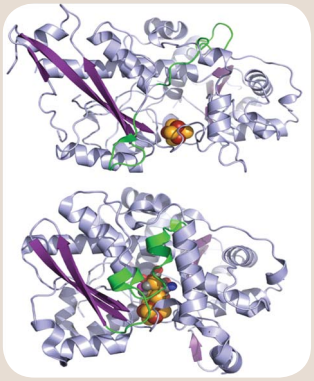
MC determines the three-dimensional (3-D) structure of biological molecules down to near atomic resolution (<1 Å), thereby helping elucidate the detailed mechanisms by which macromolecules carry out their functions in living cells and organisms. MC stations provide high-intensity beams for MAD/SAD and monochromatic data collection and also offer highly automated robotics-based, high-throughput crystal screening and data collection. All MC beamlines provide remote access control, allowing users to perform measurements from their home institutions. A state-of-the-art microbeam station with a high-performance, large-area pixel array detector (PAD) enables study of the most challenging biomolecular problems (e.g., large macromolecular complexes with large unit cells, small "micro" crystals, and mechanically and radiation-sensitive samples).

XAS is used to obtain structural information on metal sites in biomolecules. The focus at the optimized XAS beamlines and instrumentation is on dilute metalloprotein XAS, microbeam imaging/XAS, low-Z XAS (for studies of ligands such as sulfur and chlorine), and polarized single-crystal XAS studies. The range of XAS equipment includes advanced solid-state array X-ray fluorescence detector systems, liquid-helium cryostats and Kirkpatrick-Baez optic micro-XAS instrumentation, and wet-laboratory facilities. The SMB Center provides software for beamline and instrumentation control, data acquisition, and on- and off-line data analysis.

SAXS features state-of-the-art experimental facilities for solution scattering, lipid membrane diffraction, fiber diffraction, and single-crystal diffraction at moderately high to very small scattering angles in lengths ranging from microns to a few angstroms. These techniques allow structural studies of biological macromolecules and assemblies in physiological or near physiological conditions, complementing high-resolution structural techniques that require experimental conditions not necessarily physiological. In addition to providing user-friendly experimental facilities for equilibrium studies, this station maintains premier experimental facilities for time-resolved studies on time scales of milliseconds and above.

SMB Center User Highlight

Assembly Path of Multi-Metal Catalysis Clusters in [FeFe]-Hydrogenases Revealed. Complex enzymes containing iron-sulfur (Fe-S) clusters are ubiquitous in nature where they are involved in a number of reactions fundamental for life, including carbon dioxide and nitrogen fixation and hydrogen metabolism. Because these enzymes have high catalytic rates of hydrogen production, their potential for improving hydrogen–fuel cell technologies is the focus of much interest. One type of such enzymes, the [FeFe]-hydrogenases, is being investigated as an alternative biological catalyst to enzymes containing precious metals such as platinum. The active site of this hydrogenase, the H-cluster, has a [4Fe-4S] subcluster bridged to a 2Fe subcluster. Advancements in understanding how this H-cluster is synthesized in nature could contribute significantly to both the genetic engineering of hydrogen-producing microorganisms and the synthesis of biomimetic hydrogen-production catalysts. X-ray crystallography data from an intermediate, not-yet-mature form of [FeFe]-hydrogenase present insights into how the H-cluster (bio)synthesis occurs.



Ribbon Diagrams of Protein Structures. **Top:** *Intermediate structure, with the [4Fe-4S] cluster in red and gold. The two conserved loop regions thought to undergo major conformational rearrangement are in green.* **Bottom:** *Native structure (Cpl) in the same orientation as the intermediate, with the intact H-cluster in red and gold. The regions of Cpl corresponding to the loop regions of the intermediate are in green. [Image reprinted by permission from Macmillan Publishers Ltd.: Mulder, D. W., et al. 2010. "Stepwise [FeFe]-Hydrogenase H-Cluster Assembly Revealed in the Structure of HydA$^{\Delta EFG}$," Nature* ***465****, 248–51.]*

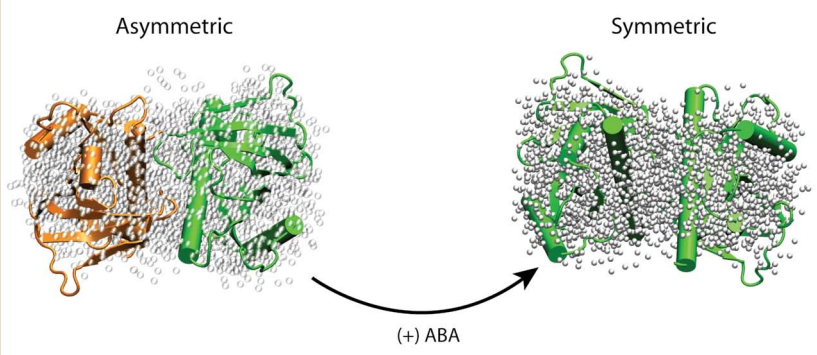
Structurally Integrated Biology for the Life Sciences (SIBYLS) Beamline

Location: *Advanced Light Source, Lawrence Berkeley National Laboratory*
Website: *sibyls.als.lbl.gov*
Principal Investigator: *John Tainer, jatainer@lbl.gov, 510-486-4158*
User Contact: *Jane Tanamachi, jtanamachi@lbl.gov, 510-495-2404*

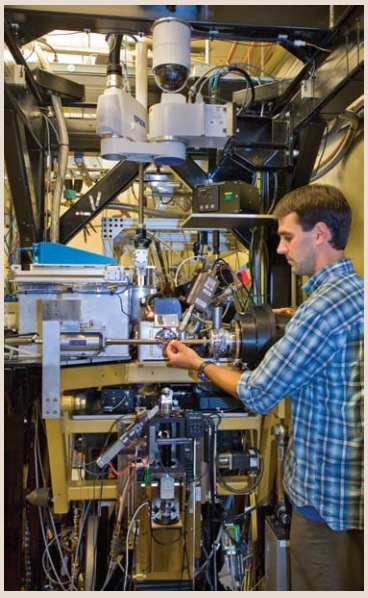
The SIBYLS beamline is a dual-end station combining macromolecular crystallography (MX) with small-angle X-ray scattering (SAXS). MX extracts high-resolution structural information from biological molecules, and the high-throughput SAXS pipeline enables the same biological systems to be imaged in aqueous solution, closer to their natural state. Combining SAXS results with atomic-resolution structures provides detailed characterizations in solution of mass, radius, conformations, assembly, and shape changes associated with protein folding and functions. SAXS also can resolve ambiguities of crystallography by showing the most likely possible structures.

SIBYLS User Highlight

Key Plant Receptors Discovered. The phytohormone abscisic acid (ABA) plays important regulatory roles in physiological pathways for plant growth and development and enables adaptation to environmental stresses, yet the protein recognition mechanisms for this hormone have eluded plant biologists. SIBYLS' crystallographic and SAXS capabilities enabled researchers to determine the atomic resolution of the ABA receptor and identify conformational changes on the ABA binding site. Elucidating the structural mechanisms mediating ABA receptor recognition and signaling is essential for understanding and manipulating abiotic stress resistance. These results were listed as one of the top 10 scientific breakthroughs of the year in 2009 by *Science*. [Nishimura, N., et al. 2009. "Structural Mechanism of Abscisic Acid Binding and Signaling by Dimeric PYR1," *Science* **326**, 1373–79.]



Elucidation of the PYR1 Protein. *Water stress in plants is communicated by the plant hormone ABA. Several proteins in plants form an ABA signaling pathway that bind the hormone and tell the plant to prepare for drought. The first step in this pathway involves the protein, PYR1 (***above***). Using X-ray diffraction (green and orange structure) and small-angle scattering (beads) experiments at the SIBYLS beamline, scientists were able to demonstrate that PYR1 undergoes a structural change upon binding ABA. The change in structure tells the next protein in the pathway that ABA is present, thereby starting the stress signaling process in plants. These experiments will undoubtedly lead to new methods for managing low-water environments for fire prevention and food production. [Image credit: Lawrence Berkeley National Laboratory]*



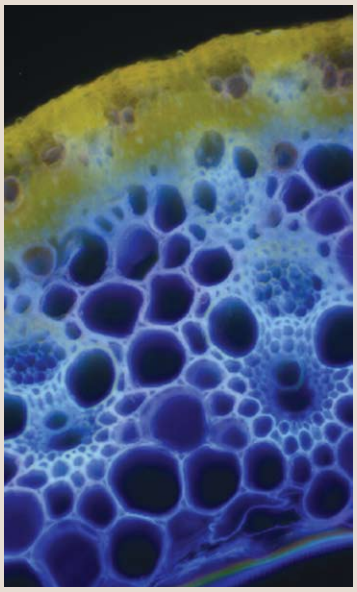
SIBYLS Beamline at the Advanced Light Source. *This experimental station allows researchers to do small-angle X-ray scattering. [Image credit: Lawrence Berkeley National Laboratory]*

Center for Structural Molecular Biology (CSMB)

Location: *High-Flux Isotope Reactor and Spallation Neutron Source, Oak Ridge National Laboratory*
Website: *www.csmb.ornl.gov*
Principal Investigator: *Dean Myles, mylesda@ornl.gov, 865-574-0548*
User Contact: *csmb@ornl.gov, 865-574-4882*

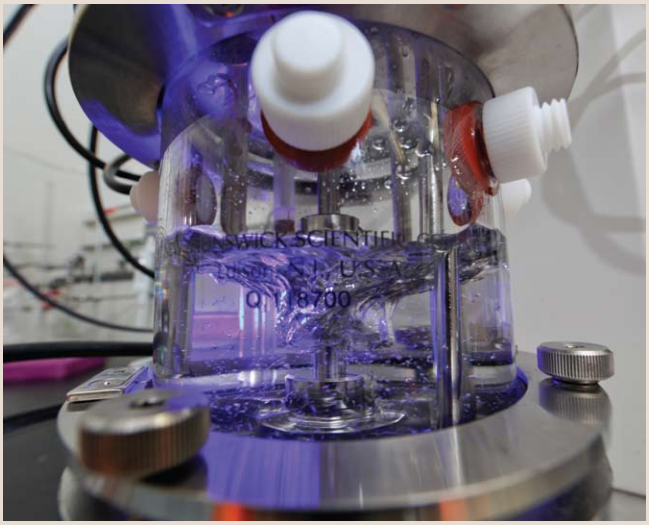
CSMB is dedicated to developing instrumentation and methods for determining the structure, function, and dynamics of complex biological systems. CSMB's suite of tools includes a small-angle neutron scattering (SANS) facility for studying biological samples under physiological (or physiologically relevant) conditions, a bio-deuteration laboratory for *in vivo* isotopic labeling, and advanced computational resources for modeling proteins and protein complexes in solution. Deuterium-labeling techniques enable scientists to selectively highlight and map chemically distinct components of larger protein-protein, protein-lipid, or protein-nucleic acid complexes and, moreover, to follow their conformational changes and assembly or disassembly processes in solution on biologically relevant time scales. These capabilities are helping researchers understand how macromolecular systems are formed and interact with other systems in living cells—ultimately bridging the information gap between cellular function and the molecular mechanisms that drive it.

CSMB User Highlight



Cryo-Electron Microscopy Image of Switchgrass Cell Wall. *Study of this biomass material by SANS revealed that although the biological cell wall architecture of switchgrass (as shown above) remains largely intact during dilute acid pretreatment, dramatic changes are occurring at the molecular level, including swelling of cellulose fibers and the redistribution of lignin into clusters. [Image credit: National Renewable Energy Laboratory and DOE BioEnergy Science Center]*

Improving Access to Cellulose in Biomass for Biofuel Production. The conversion of cellulosic biomass to fermentable sugars usually requires costly, time-consuming pretreatment to increase the material's porosity, decrease its crystallinity, and reduce the amount of structural lignin in the cell wall. Researchers used SANS to probe the morphological changes of switchgrass cell walls during dilute acid pretreatment, elucidating the interplay of different biomolecular components in the breakdown process. The results are important for the development of efficient strategies to convert biomass to biofuel. [Pingali, S. V., et al. 2010. "Breakdown of Cell Wall Nanostructure in Dilute Acid Pretreated Biomass," *Biomacromolecules* **11**, 2329–35.]



CSMB Bio-Deuteration Laboratory. *The technique of bio-deuteration is particularly effective in highlighting specific parts of target molecules, such as reaction centers, and in distinguishing the presence of labeled molecules incorporated in larger biological complexes or assemblies. [Image credit: Oak Ridge National Laboratory]*

Advanced Biological and Environmental X-Ray Spectroscopy (ABEX)

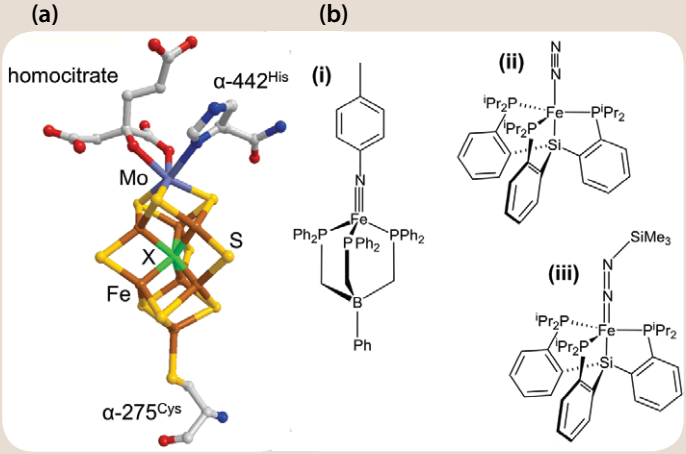
Location: *Advanced Light Source, Lawrence Berkeley National Laboratory*
Website: *abex.lbl.gov*
Principal Investigator: *Stephen Cramer, spcramer@lbl.gov, 510-486-4720*

ABEX is a new user resource that enables X-ray spectroscopic characterization of complex biological and environmental systems using soft X-ray absorption (XAS), X-ray magnetic circular dichroism (XMCD), and resonant inelastic X-ray scattering (RIXS). These spectroscopies exploit the availability of high brightness, circularly polarized soft X-rays at the Advanced Light Source and offer unique advantages in analyzing the detailed electronic and magnetic structure of biological metal sites.

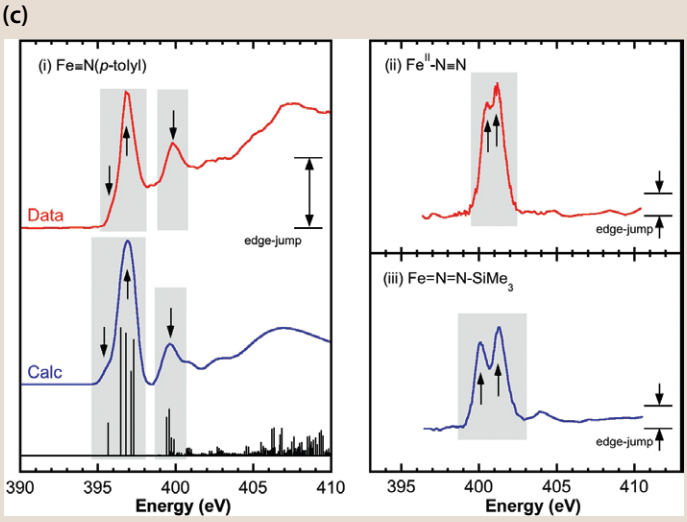
ABEX also has a major instrument development program to improve both the sensitivity and user-friendliness of its instruments. A spectroscopy support laboratory will provide electron paramagnetic resonance (EPR), infrared, and resonance Raman spectroscopies, enabling essential control measurements on the same samples studied by the X-ray techniques.

ABEX User Highlight

Revealing the Molecular Underpinnings of a Key Enzyme. As a major component of the biological nitrogen cycle, the bacterial enzyme MoFe nitrogenase converts nitrogen from air into ammonia, thereby making it accessible to plant life. The enzyme achieves this feat at a metal-sulfur cluster called FeMo-co, the molecular mechanism of which is not well understood. Research to better understand how metals and metal clusters interact with nitrogen and reduced nitrogen species is exploiting the soft X-ray region and using nitrogen K-edge spectroscopy to study a series of specially synthesized model compounds. Each system is designed to exhibit different modes of binding nitrogen and dinitrogen [see figures (a) and (b)]. The spectra are striking, with intense pre-edge features arising from the multiple bonded nitrogen species, which in turn reveal unique information about the bonding interactions of nitrogen species with the metal site [see figure (c)]. These results, along with theoretical calculations, are further enhancing understanding of the chemistry that underpins this biological process. [Paper in preparation.]



Molecular Structures. **(a)** *FeMo-co and* **(b)** *examples of metal-nitrogen model systems. [Image credits: Lawrence Berkeley National Laboratory]*



Spectroscopy Results. *Nitrogen K-edge spectra measured on metal-nitrogen model systems. [Image credits: Lawrence Berkeley National Laboratory]*

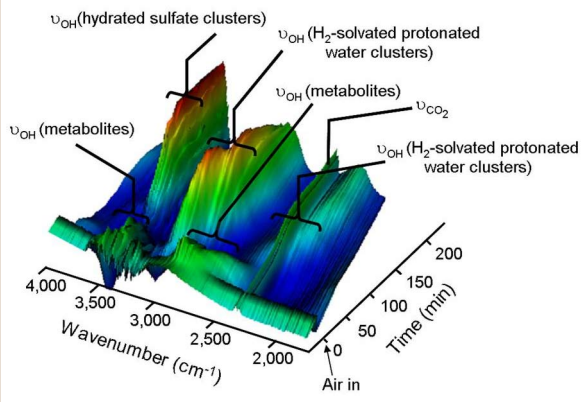
Berkeley Synchrotron Infrared Structural Biology (BSISB) Program

Location: *Advanced Light Source, Lawrence Berkeley National Laboratory*
Website: *infrared.als.lbl.gov*
Principal Investigator: *Hoi-Ying Holman, hyholman@lbl.gov, 510-486-5943*
User Contact: *Theresa Pollard, tapollard@lbl.gov, 510-486-6740*

BSISB features synchrotron radiation–based Fourier transform infrared (SR-FTIR) microscopy, which is a label-free, noninvasive molecular technique that couples the high brightness of synchrotron radiation with the high throughput and vast analytical capabilities of FTIR spectrometers. With a synchrotron source, BSISB's FTIR microscopes are capable of diffraction-limited chemical imaging with signal-to-noise ratios 100 to 1000 times greater than standard blackbody sources.

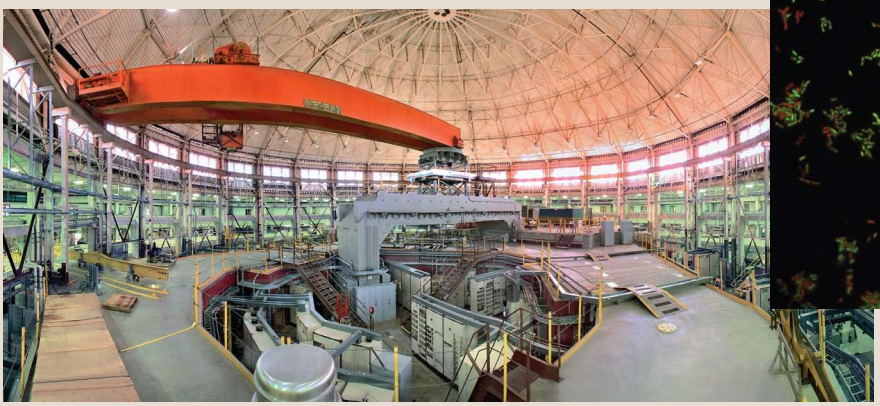
Aqueous environments hinder SR-FTIR's sensitivity of bacterial activity, but the recent development of integrated *in situ* open-channel microfluidic culturing systems circumvents the water-absorption barrier. These systems enable real-time chemical imaging of bacterial activities in biofilms and facilitate comprehensive understanding of structural and functional dynamics in a wide range of microbial systems.

BSISB User Highlight

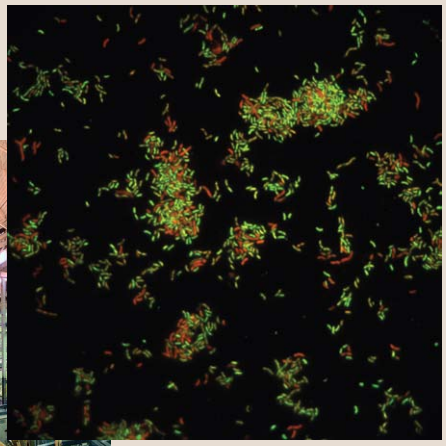


Dynamic Measurements. *Three-dimensional diagram depicting the changing chemistry inside* D. vulgaris *cells during adaptive response. [Image credit: Lawrence Berkeley National Laboratory]*

Measuring Chemical Changes Inside Living Cells. Understanding how microbes adapt to changing chemical environments is a critical aspect of using them to solve DOE challenges. With SR-FTIR, researchers tracked the chemistry of living *Desulfovibrio vulgaris* cells in real time. The ability to make these dynamic measurements continuously inside selected living cells dramatically increases the usefulness and reliability of information traditionally derived from cells that have been killed and broken apart. [Holman, H.-Y., et al. 2009. "Real-Time Molecular Monitoring of Chemical Environment in Obligate Anaerobes During Oxygen Adaptive Response," *Proceedings of the National Academy of Sciences (USA)* **106**, 12599–604.]



BSISB Experimental Station. *FTIR instrumentation is located at the back just to the right of center (above) at the Advanced Light Source. [Image credit: Lawrence Berkeley National Laboratory]*



Imaging Cells. *Live (green stain) and dead (red stain) image of* D. vulgaris *cells during air exposure. [Image credit: Lawrence Berkeley National Laboratory]*

National Center for X-Ray Tomography (NCXT)

Location: *Advanced Light Source, Lawrence Berkeley National Laboratory*
Website: *ncxt.lbl.gov*
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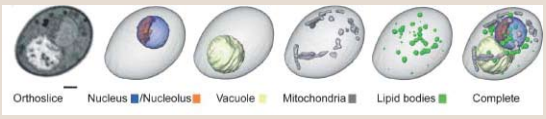
User Contact: *Mark Le Gros, malegros@lbl.gov, 510-486-6892*

A primary research area at NCXT is development of soft X-ray tomography (SXT), a technique for quantitatively imaging whole, hydrated cells in 3-D. This technique has several distinct advantages over light and electron microscopy and is contributing unique insights on cells and their behavior. The soft X-ray illuminating photons used in SXT penetrate biological materials much more easily than electrons and allow specimens up to 10 µm thick to be imaged. Unlike electron microscopy, SXT eliminates the need to section eukaryotic cells with an ultramicrotome before imaging. Since contrast in SXT is produced directly by the differential absorption of X-rays, specimens do not have to be dehydrated or stained. Consequently, SXT produces high-resolution views of specimens in a near-native state.

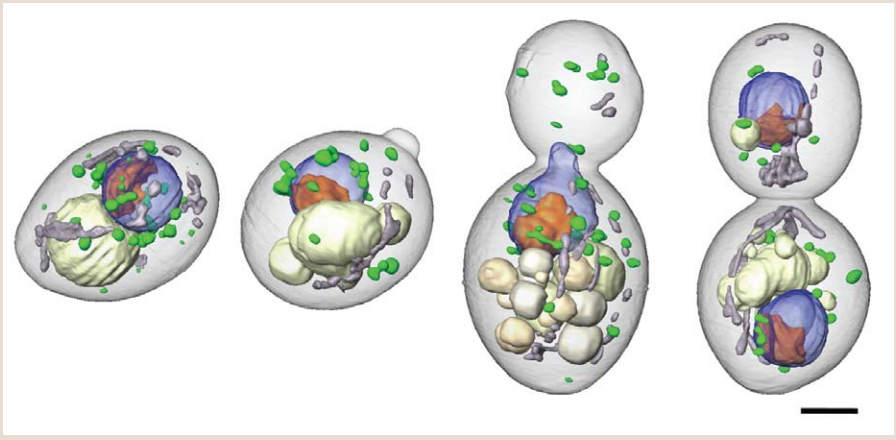
SXT has become an even more powerful technique because of the development of high-aperture cryogenic light microscopy for correlated imaging. This multimodal approach allows labeled molecules to be localized in the context of a high-resolution, 3-D tomographic reconstruction of a cell.

NCXT User Highlight

Monitoring Cell Size and Organelle Volumes in Yeasts. Cell size is a key factor in initiating cell division in yeasts, and the number and volume of organelles have a profound impact on the function and viability of a cell. SXT was used to characterize these parameters in strains of *Saccharomyces cerevisiae* at each of the key stages in the cell cycle and to determine relationships between cellular and organelle volumes. Results showed that growth of the major organelles—with the notable exception of vacuoles—is strictly regulated in accordance with cell size. Similar ratios were found to be maintained in *Schizosaccharomyces pombe* and *Candida albicans*. These experiments will undoubtedly improve our understanding of how cells control their size and that of their component organelles. [Uchida, M., et al. 2011. "Quantitative Analysis of Yeast Internal Architecture Using Soft X-Ray Tomography," *Yeast* **28**, 227–36.]



Organelle Segmentation. *Representative diploid cell shown in an orthoslice (i.e., a single slice of tomographic data) and individually segmented organelles; scale bar = 1 µm. [Image credit: Lawrence Berkeley National Laboratory]*



Tomographic Cell Reconstruction. *Representative diploid* S. cerevisiae *cells at the four main stages of the cell cycle; scale bar = 1 µm. [Image credit: Lawrence Berkeley National Laboratory]*

For More Information

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U.S. Department of Energy Office of Science

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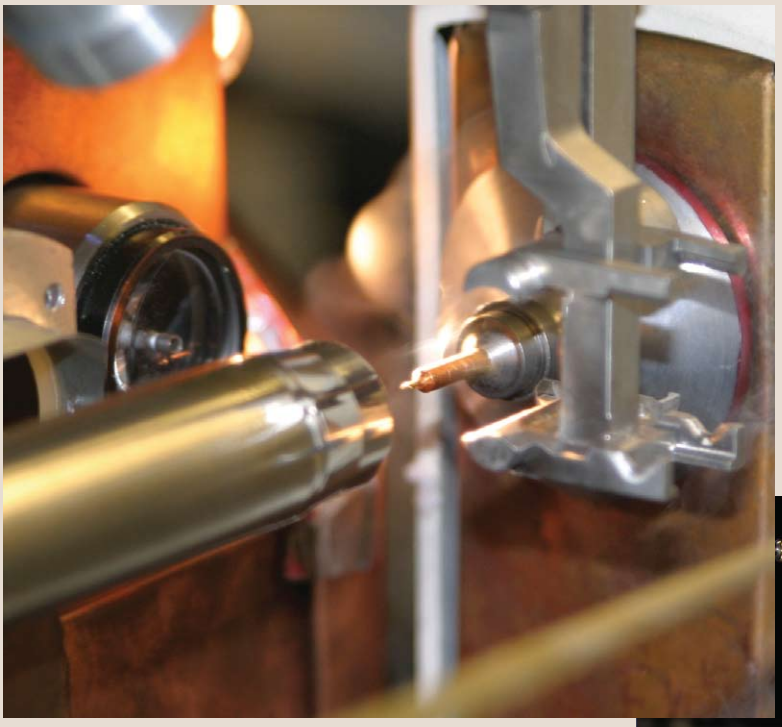
Genomic Science (*science.energy.gov/ber/research/bssd/genomic-science/*)

Structural Biology (*science.energy.gov/ber/research/bssd/structural-biology/*)

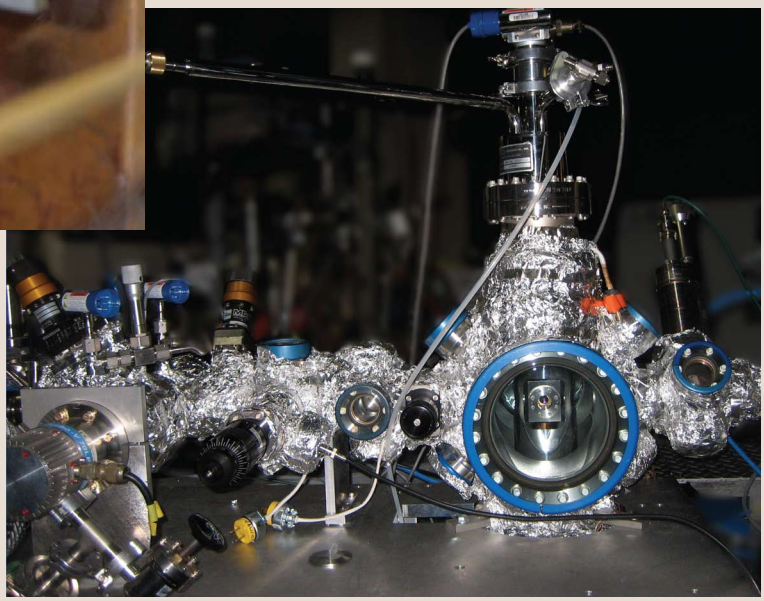
DOE Office of Biological and Environmental Research (*science.energy.gov/ber/*)

DOE Office of Science (*science.energy.gov*)

U.S. Department of Energy (*energy.gov*)



Robot in action at Stanford Synchrotron Radiation Lightsource. [Image credit: SLAC National Accelerator Laboratory]



A helium atmosphere soft X-ray chamber being developed for the Advanced Biological and Environmental X-Ray Spectroscopy experimental station at the Advanced Light Source. [Image credit: Lawrence Berkeley National Laboratory]

May 2011