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New Structural Biology Facilities at NSLS-II

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The National Synchrotron Light Source II (NSLS-II), a Department of Energy (DOE) Office of Science User Facility located at Brookhaven National Laboratory, offers an advanced suite of beamlines dedicated to structural biology research for investigating problems ranging from the atomic to the cellular scale. The beamlines are now available for general users, while adjacent wet and dry laboratories will become available shortly.

These beamlines offer X-ray diffraction, scattering, footprinting, spectroscopy, and imaging techniques that capitalize on NSLS-II's brightness.

Crystallography beamlines

NSLS-II offers three macromolecular crystallography (MX) beamlines with undulator sources. The Highly Automated Macromolecular Crystallography (AMX) beamline enables MX with a beam size as small as 5 µm for structure determination from the most challenging crystals, especially of large complexes, ribosome, and membrane proteins. Measurements on hundreds of microcrystals, automated and unattended data collection for ligand binding studies, and room-temperature data collection from compact *in situ* crystallization trays are all possible.

The Frontier Microfocusing Macromolecular Crystallography (FMX) beamline supports MX with a beam size from 1 to 10 µm for structure determination from difficult-to-measure large crystals and from the smallest crystals, such as of G-Protein Coupled Receptors (GPCRs) that are important targets in drug research. FMX embraces ultra-fast scanning and crystal-jet serial crystallography, the latter via collaboration with Arizona State University (ASU), to obtain complete datasets from hundreds to thousands of microcrystals that survive only a few milliseconds in the bright beam.

The Biological Microdiffraction Facility (NYX) beamline is operated by the New York Structural Biology Center (NYSBC) in partnership with NSLS-II. The beamline's tunable optics deliver very fine energy resolution ($\Delta E/E = 5 \times 10^{-5}$) to optimize anomalous diffraction experiments across a broad spectrum of atomic resonances from biologically relevant elements.

Scattering beamline

The Life Science X-ray Scattering (LiX) undulator beamline addresses protein solution scattering using simultaneous collection of small- and wide-angle scattering data that typically span a continuous q range of 0.005–3.2 $Å^{-1}$, and instantaneous switching between static measurements and in-line size exclusion chromatography. LiX also upholds scanning scattering measurements, using beam sizes of a few µm, for analyzing structures in biological tissues such as cell wall cellulose in plant sections and myelin in brain tissues. A joint program established with Oak Ridge National Laboratory allows access to both LiX and the Bio- Small Angle Neutron Scattering instrument at the High Flux Isotope Reactor facility via a single beamtime proposal.

Footprinting/spectroscopy beamline

The Biological X-ray Footprinting and Spectroscopy (XFP) three-pole wiggler (3PW) beamline is operated by Case Western Reserve University (CWRU) in partnership with NSLS-II. For footprinting, a focused white beam enables sub-millisecond exposures for insight into macromolecular structure and dynamics of solution samples in near-physiological to *in vivo* conditions with single-

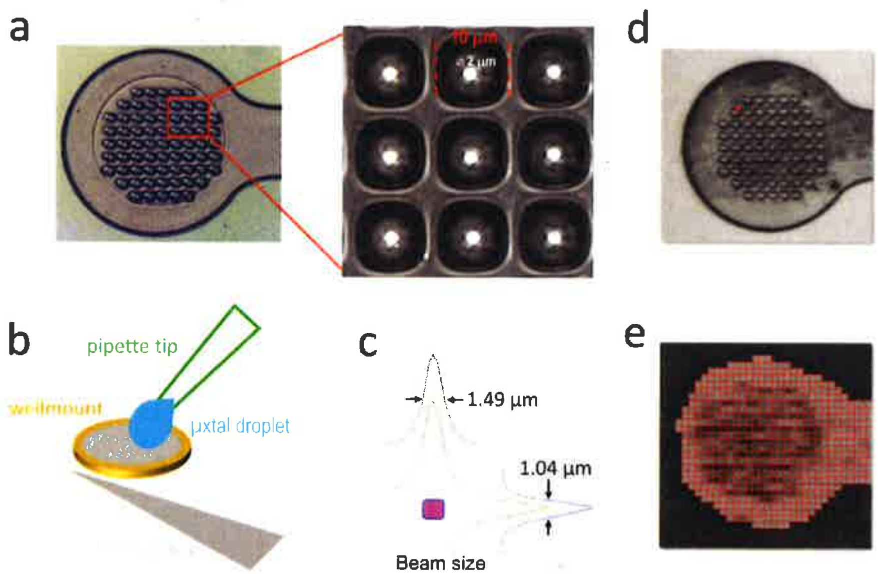

Figure 1: Manipulation of microcrystals for micro-crystallography. (a) Patterned well mount. Inset: A high-resolution image of microwells to show their shape and dimensions. For better visualization, the 2 µm holes are highlighted by white circles. (b) A generic procedure for harvesting and cooling microcrystals. A micropipette is used to aspirate microcrystals and the microcrystal droplet is deposited onto the top side of the well mount; solvent is then removed from the bottom side by using a filter paper and the well mount is plunged into liquid nitrogen for cooling. (c) The 1.0 × 1.5 µm FMX beam profile measured at 12.6 keV. (d) A light microscope view of a well mount loaded with microcrystals in an orientation ready for raster scanning. (e) A raster-scan heat map of diffraction intensity from microcrystals on a well mount. Reproduced with permission courtesy of G. Guo et al., IUCrJ 5, 238–246 (2018).

amino-acid or single-nucleotide resolution. For spectroscopy, a tunable monochromator accesses absorption edges of elements associated with metalloproteins and bio-inspired homogeneous catalysts, offering insights into metal site structure and oxidation state for samples not amenable to crystallization, such as trapped reactive intermediates.

Imaging and microscopy beamlines

Several beamlines enable microscopy and spectroscopy studies in a wide range of spatial resolutions for many elements across the periodic table important for bio-imaging. The X-ray Fluorescence Microprobe (XFM) 3PW beamline provides biologists with unique tools for characterizing elemental abundances and chemical speciation in organisms, tissues, and cells, and is optimized for mesoscale imaging and spatially resolved X-ray absorption spectroscopy at the micron scale.

In addition, the Hard X-ray Nanoprobe (HXN) undulator beamline delivers world-leading 10 nm spatial resolution using multilayer Laue lenses. The Submicron Resolution X-ray Spectroscopy (SRX) undulator beamline offers hard X-ray spectromicroscopy capabilities at sub-micron resolution, while the Tender Energy Spectroscopy (TES) bending magnet beamline is optimized for interrogation of lighter elements, including phosphorus, sulfur, and calcium at the micron scale. The Full Field X-ray Imaging (FXI) multipole wiggler beamline hosts a transmission X-ray microscope with 30 nm resolution.

Collaborations across Brookhaven Lab

Collaborations are underway with Brookhaven's Biology Department to develop serial crystallography methodology (see Figure 1 for an example using FMX), the Quantitative Plant Science Initiative for structure determination of proteins involved in plant metabolism, and the Center for Functional Nanomaterials to develop sample holders.

New CryoEM and future outlook

A cryo-electron microscopy (cryoEM) suite is being established close to the beamlines and laboratories, allowing for sample preparation and high-resolution structure determination. The suite is expected to open to users in 2019.

As the NSLS-II facility matures, overlap between the advanced structural biology and imaging beamlines is developing, leading to the possibility of coordinated multidisciplinary research. Several themes are emerging: increased use of X-ray microbeams for serial crystallography, automated data analysis, multidisciplinary scattering experiments, and imaging of the cellular environment. Our task is now to enable these themes to be combined to allow for sophisticated scientific questions to be posed and answered.

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