X-RAY FOOTPRINTING FOR *IN VITRO* AND *IN VIVO* STRUCTURAL STUDIES OF BIOLOGICAL MACROMOLECULES (XFP)

BROOKHAVEN NATIONAL LABORATORY

NATIONAL SYNCHROTRON LIGHT SOURCE II

SCIENTIFIC SCOPE

X-ray Footprinting for *in Vitro* and *in Vivo* Structural Studies of Biological Macromolecules (XFP) is a 3-pole wiggler beamline at 17-BM providing world-leading X-ray Footprinting capabilities for solution state studies of biological macromolecular structure and dynamics. The focused broadband X-ray beam delivers flexible beam spot sizes to adapt to sample configurations; the high flux density of the XFP beam enables achievement of exposure times of less than a millisecond for high quality data on complex samples in physiologically relevant conditions and timescales.

BEAMLINE CHARACTERISTICS

TECHNIQUES:

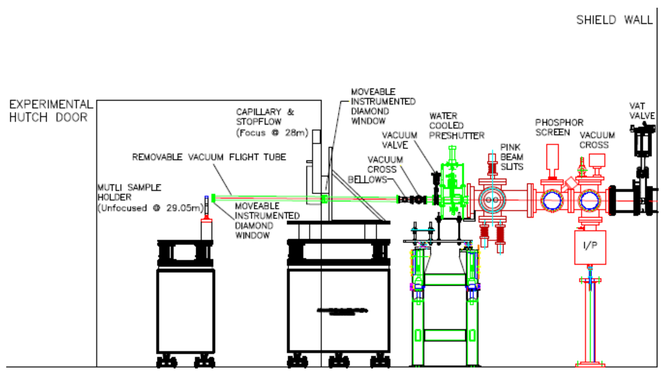
- X-ray Footprinting

ENDSTATION DETAILS:

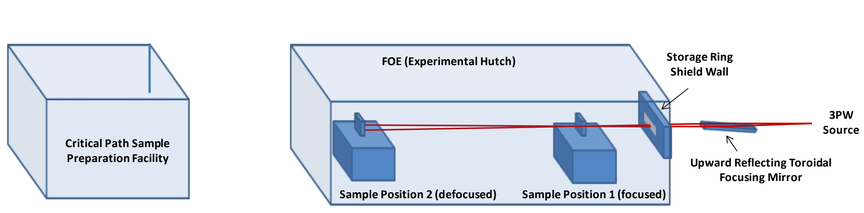
- High-throughput Automated Steady State Apparatus
- Quench-flow Apparatus for Time-resolved Measurements
- Capillary Flow Cell for High Flux or *in Vivo* Experiments

XFP at NSLS-II:

- Structural studies of complex macromolecules in solution state
- Single amino acid and/or single nucleotide resolution
- Near-physiological to *in vivo* conditions
- Rapid exposure for time-resolved measurements on biologically relevant timescales (microseconds to minutes)



XFP Endstation
Two sample positions provide flexible beam sizes to adapt to diverse sample configurations.



XFP Beamline Schematic
Single toroidal focusing mirror located inside the front end; experiments are performed in the first optical enclosure. A sample preparation area at the beamline enables study of sensitive and unstable samples.

Overview

PORT: 17-BM
SOURCE: 3-Pole Wiggler (3PW)
ENERGY RANGE: 5 – 20 keV (broadband)
FLUX DENSITY: >500 W/mm^2 (at focus)
CONSTRUCTION PROJECT: Partner – Case Western Reserve University/NSF
BEAMLINE STATUS: Construction
AVAILABLE TO USERS: Spring 2016

Beamline Team

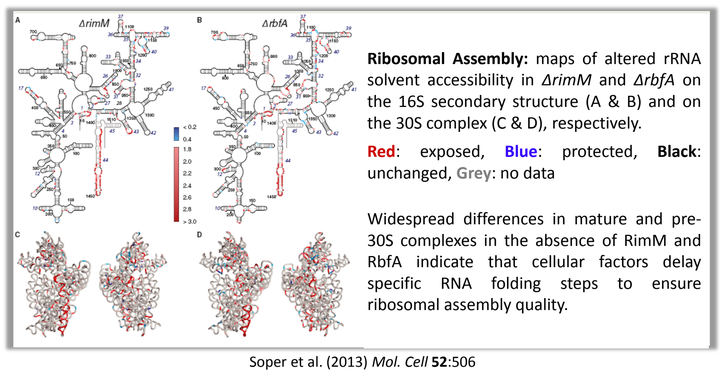
STAFF

Mark R. Chance: spokesperson
Jen Bohon: lead beamline scientist
Michael Sullivan: beamline engineer
Don Abel: technician
John Toomey: technician
Howard Robinson: PSD project manager

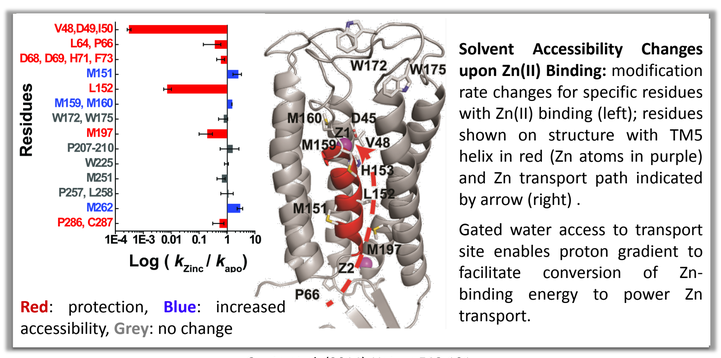
ADVISORS

Sarah Woodson (Johns Hopkins University)
Gwyn Williams (Jefferson Lab)
Corie Ralston (Lawrence Berkeley Nat'l Lab)
Steve Almo (Albert Einstein College of Medicine)

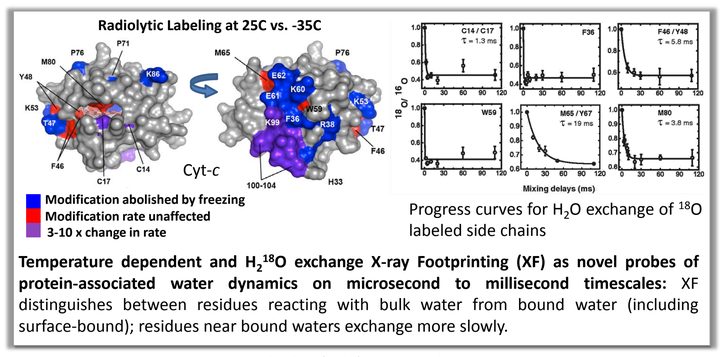
SCIENTIFIC APPLICATIONS



Soper et al. (2013) *Mol. Cell* **52**:506



Gupta et al. (2014) *Nature* **512**:101.



Gupta et al. (2012) *PNAS* **109**: 14882.

In Vivo Studies

Reduction of exposure times *in vivo* to the range of single digit milliseconds will reduce sample damage due to overexposure.

Shown above: *in vivo* study of 30S ribosome assembly.

Membrane Protein Dynamics

The high flux density beam at XFP will overcome the effect of strong hydroxyl radical scavengers in membrane fractions & liposomes.

Shown above: mechanism of proton-coupled zinc transport.

Water Dynamics

Microsecond scale exposure times at XFP will enable detailed observation of dynamics of water interactions with macromolecules.

Shown above: proof of principle XF study of water dynamics.

SCHOOL OF MEDICINE CASE WESTERN RESERVE UNIVERSITY

This work receives support from NSF DBI-1228549, NSF DBI-1255340 and NIBIB P30-EB-009998.