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:

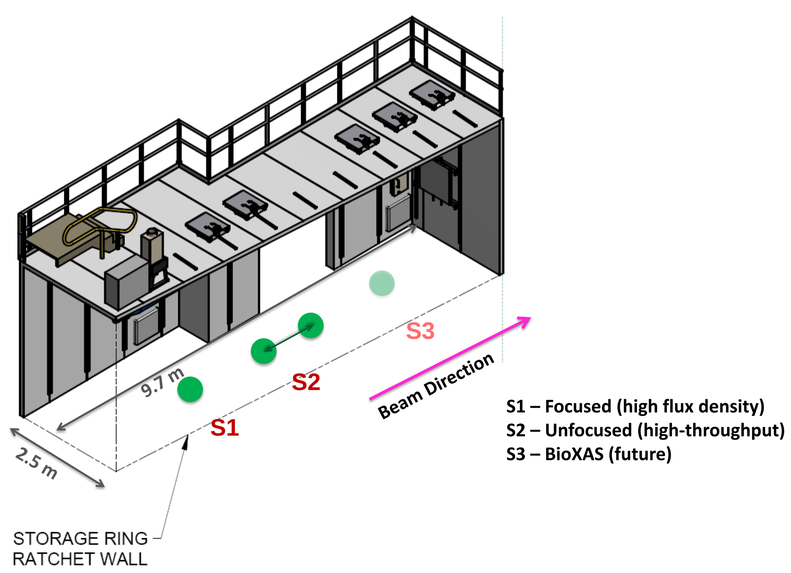
- Steady-state X-ray Footprinting (capillary flow) with minimum beam size of 120 µm x 450 µm FWHM (>200 W/mm^2 max power density) and minimum exposure time of 75 µs
- High-throughput X-ray Footprinting for low volume (5 µL) , liquid or frozen state (-30 C) samples; beam size ~2.6 mm diameter FWHM; minimum exposure time of 10 ms
- Time-resolved X-ray Footprinting (capillary flow) with beam parameters as defined by capillary flow; enables ms scale mixing times and 100 µs scale exposures

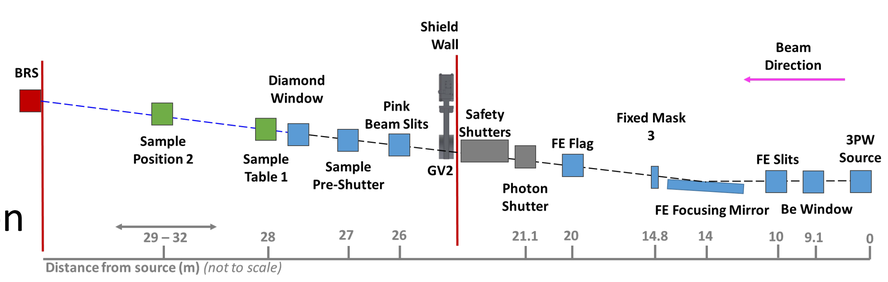
ENDSTATION DETAILS:

- Syringe pump driven capillary flow cell for high flux density experiments and *in vivo* studies at beam focal position; fraction collector available
- High-throughput automated steady state apparatus (frozen or RT) with unfocused beam
- Mixing apparatus for time-resolved measurements at beam focal position
- Focused beam imaged via kilopixel transmission diamond detector

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)





Overview

PORT: 17-BM

SOURCE: 3-Pole Wiggler (3PW)

ENERGY RANGE: 5 – 16 keV

ENERGY RESOLUTION: broadband

BEAM SIZE: 120 x 450 µm^2 (at focus)

POWER DENSITY: >200 W/mm^2 (at focus)

BEAMLINE STATUS: Open to users

BEAMLINE PHONE: 631.344.1817

BEAMLINE WEBSITE: www.bnl.gov/nsls2/beamline/17-BM

PARTNER BEAMLINE: Case Western Reserve University

Beamline Staff

Jen Bohon
Lead Beamline Scientist

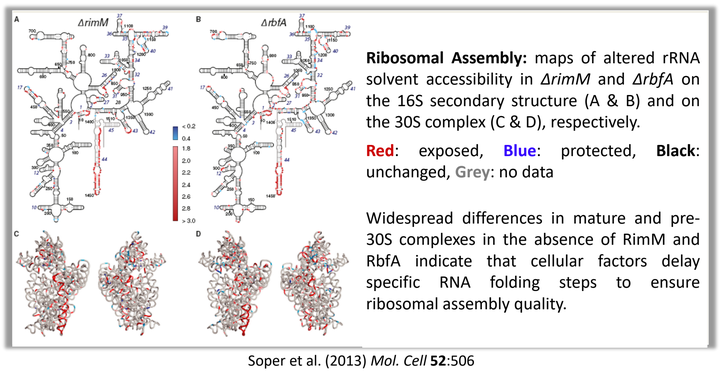
Erik Farquhar
Beamline Scientist

Apply for Beam Time

Beamlines at NSLS-II are available to all scientific researchers through a peer-review proposal process. For more information, see the NSLS-II Users' Guide at:

www.bnl.gov/ps/userguide/

SCIENTIFIC APPLICATIONS



Ribosomal Assembly: maps of altered rRNA solvent accessibility in *ΔrimM* and *ΔrbfA* on the 16S secondary structure (A & B) and on the 30S complex (C & D), respectively.

Red: exposed, **Blue**: protected, **Black**: unchanged, **Grey**: no data

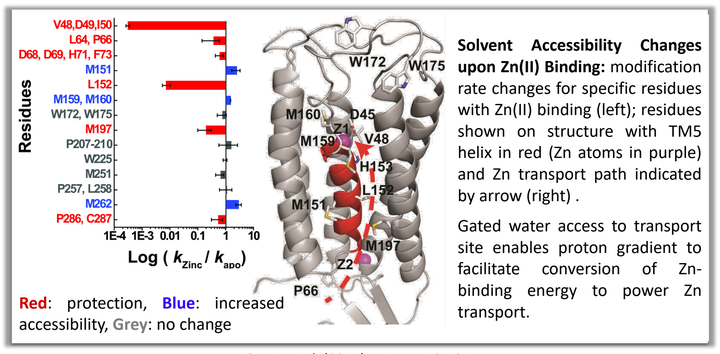
Widespread differences in mature and pre-30S complexes in the absence of RimM and RbfA indicate that cellular factors delay specific RNA folding steps to ensure ribosomal assembly quality.

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

In Vivo Studies

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

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



Solvent Accessibility Changes upon Zn(II) Binding: modification rate changes for specific residues with Zn(II) binding (left); residues shown on structure with TM5 helix in red (Zn atoms in purple) and Zn transport path indicated by arrow (right) .

Gated water access to transport site enables proton gradient to facilitate conversion of Zn-binding energy to power Zn transport.

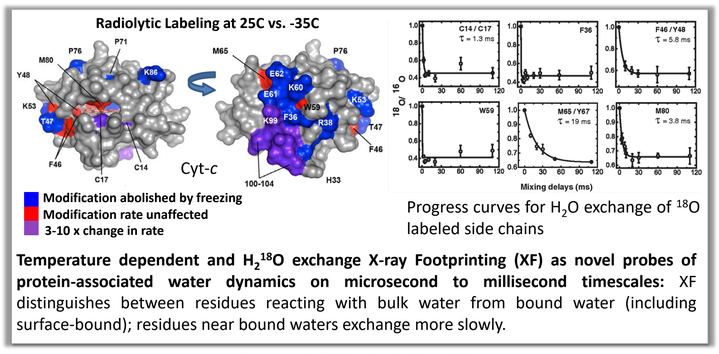
Red: protection, **Blue**: increased accessibility, **Grey**: no change

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

Membrane Protein Dynamics

High flux density beam at XFP to overcome the effect of strong hydroxyl radical scavengers in membrane fractions, organelles & liposomes

Shown above: mechanism of proton-coupled zinc transport



Progress curves for H_2O exchange of ^{18}O labeled side chains

Temperature dependent and $H_2{}^{18}O$ exchange X-ray Footprinting (XF) as novel probes of protein-associated water dynamics on microsecond to millisecond timescales: XF distinguishes between residues reacting with bulk water from bound water (including surface-bound); residues near bound waters exchange more slowly.

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

Water Dynamics

Microsecond scale exposure times to 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

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