Commissioning of the XFP Beamline for X-ray Footprinting at NSLS-II

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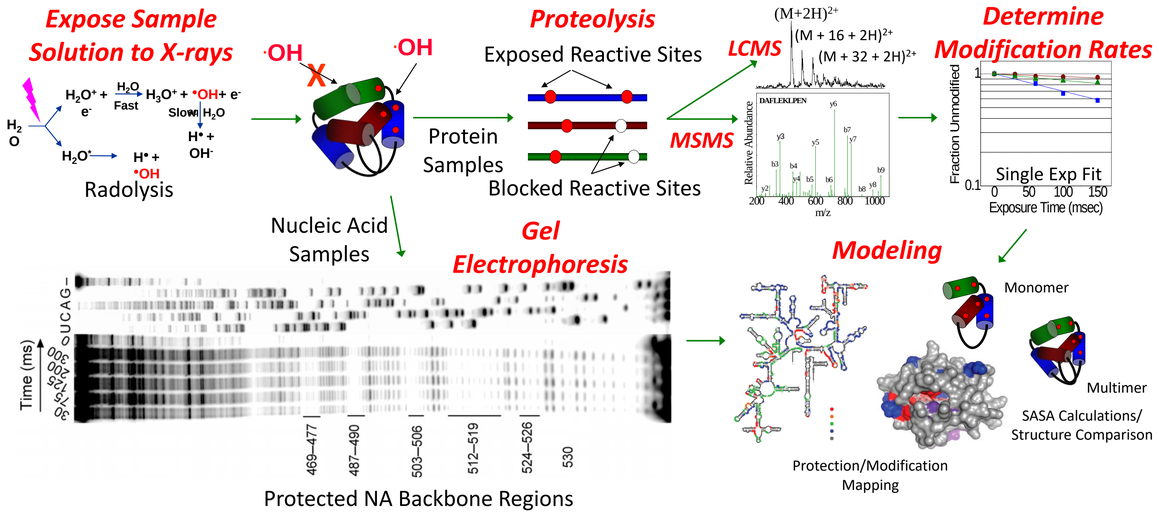
ABSTRACT

X-ray mediated hydroxyl radical footprinting coupled to mass spectrometry (MS) has developed into a powerful method for the analysis of solution-state structure and dynamics of macromolecules. This technology employs the very intense and ionizing broadband x-ray beams produced by synchrotron radiation to generate hydroxyl radicals in solution on the microsecond timescales appropriate for probing macromolecular dynamics while minimizing sample perturbation. A new resource dedicated to this technique has been developed and undergone commissioning at the premier synchrotron facility in the United States, the National Synchrotron Light Source II. This novel resource, the XFP beamline, provides unprecedented x-ray power density, enabling study of complex biological systems both *in vitro* and *in vivo*, and is now available to the scientific community. Initial beamline performance indicates a dramatic increase in the available flux density and science commissioning experiments have already shown that sub-millisecond sample exposures are easily accessible.

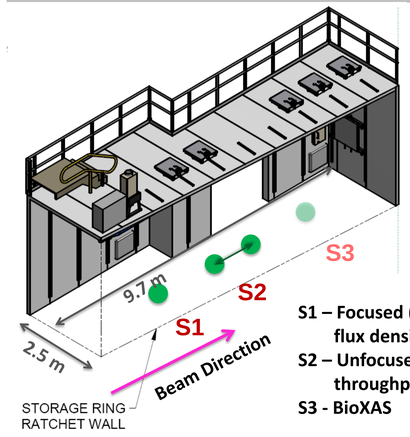
Beamline Technique

Synchrotron X-ray Footprinting

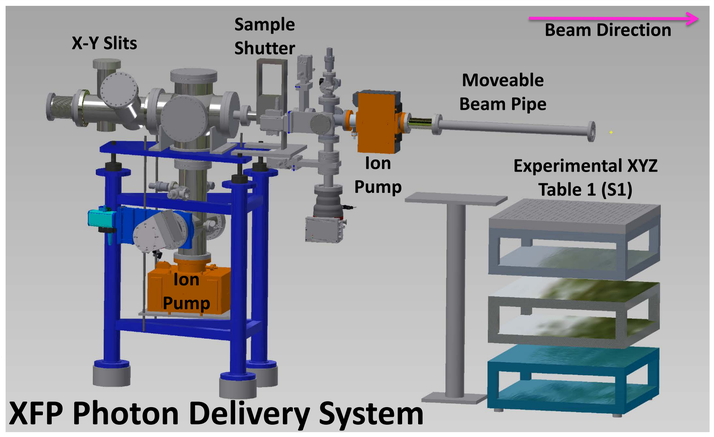


Dilute solutions of protein(s) and/or nucleic acids are exposed (ms time scale) to X-ray beams at the synchrotron, generating a transient burst of OH radicals in the solution. The radicals covalently modify solvent-exposed reactive amino acid side-chains and cleave exposed nucleic acid backbone regions, but buried residues are protected. Nucleic acids are analyzed via gel electrophoresis. Proteins are digested with proteases and analyzed via LCMS and MSMS for quantitation and verification of the modified residues. Dose-dependent plots are created to determine the rate constants for each modification. Protection maps can be generated for both proteins and NA and a structural model generated (detail level determined in part by complementary structural data). With an appropriate exposure apparatus, time-resolved footprinting data enables observation of structural dynamics.

17-BM Beamline Design

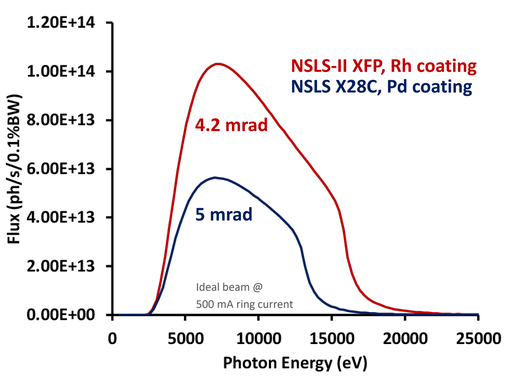


S1 – Focused (high flux density)
S2 – Unfocused (high-throughput)
S3 - BioXAS



The XFP beamline (NSLS-II 17-BM) delivers focused (single focusing mirror inside the shield wall) white beam to several sample locations and endstations in the experimental hutch. A sample preparation area is located proximal to the beamline. Construction of the BioXAS endstation is expected to begin Summer 2017.

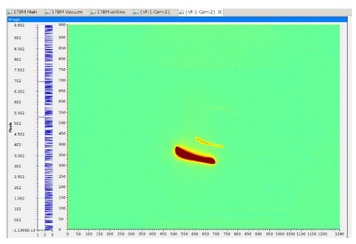
Source & Optics



Source	Hor. Size, Div. [µm, µrad]	Vert. Size, Div. [µm, µrad]
NSLS BM X28C	σ_h = 260, σ_h' = 300	σ_v = 57, σ_v' = 11
NSLS-II 3PW	σ_h = 167, σ_h' = 98	σ_v = 12.3, σ_v' = 0.82

- 3 mrad x 0.33 mrad radiation from a 3PW
- 1.1 m Rh-coated toroidal mirror at 14 m for 1:1 focus in the hutch
- 4.2 mrad fixed angle, bendable for focusing
- 5 x 1016 ph/s, 5~16 keV broadband beam

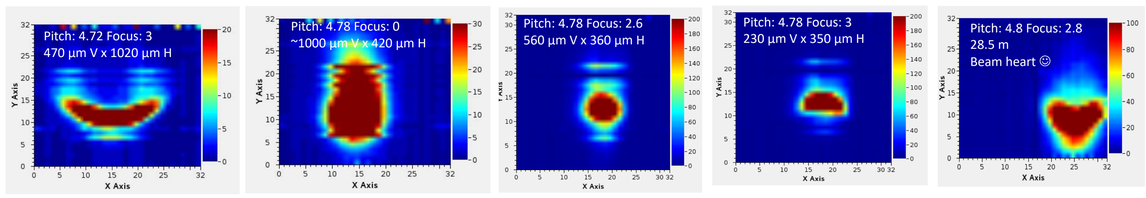
Beamline Performance



XFP first light July 2016!

XFP first opens its shutters to allow light into the front end (left) on July 10, then into the experimental hutch (right) on July 11. Beam is shown on phosphor screens.

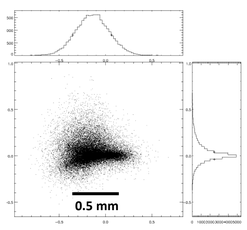
Mirror Commissioning



Beam Images from Kilopixel Imaging Detector at XFP

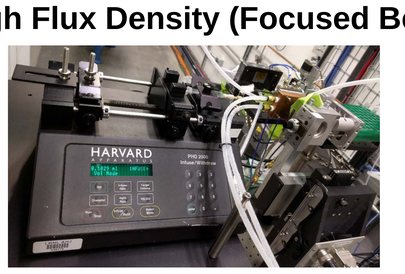
The XFP mirror was aligned using a unique kilopixel diamond imaging detector developed in house (NSF: CWRU/SBU/BNL) for this purpose (Zhou et al. (2015) JSR 22 1396). A variety of beam shapes and sizes are achievable with mirror adjustment; ongoing iterations of adjustment continue to improve performance. Several examples of beam parameters are shown above. Pitch is in mrad. Axes are 60 µm channels, color scale is arbitrary based on gain, horizontal stripes are artifacts.

High Flux Density (Focused Beam)



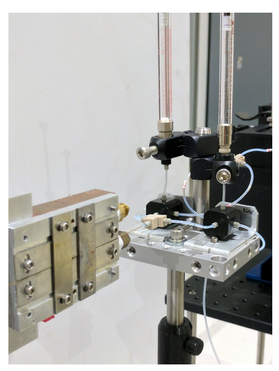
Simulation of Ideal Beam

- **80 x 425 µm FWHM**
- **>500 W/mm² (at 500mA)**

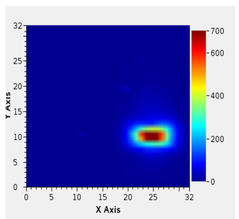


Syringe Pump and Capillary Flow Cell

- **Sample position S1**
- **200 µm Capillary**
- **Vertical flow**

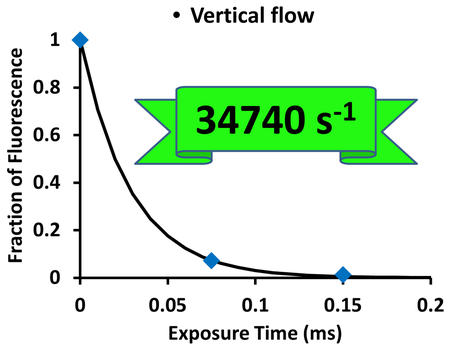


Time-resolved Apparatus



Measured Beam Focus

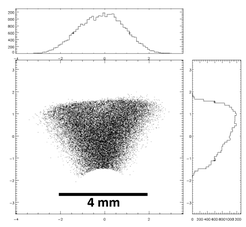
- **120 x 450 µm FWHM**
- **220 W/mm² (at 250mA)**



Record Dose Rate at XFP

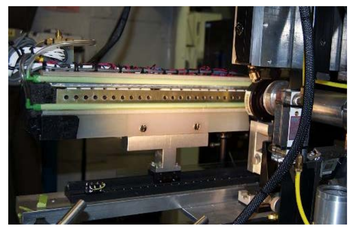
Alexa 488 dose response (left) for focused beam with 76 µm of Al attenuation (10 mM Na Phosphate pH 7.4). Previous record at NSLS X28C was 2243 s^{-1}, minimum exposure of ~300 µs – XFP has achieved >30000 s^{-1} with 75 µs exposures!

High-throughput (Unfocused Beam)



Simulation of Ideal Beam

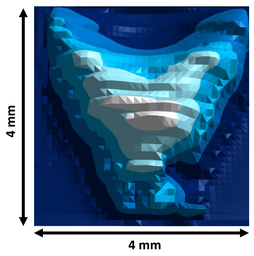
- **2.6 x 2.6 mm FWHM**
- **~12 W/mm² (at 500mA)**



Multi-sample holder

(Includes frozen samples)

- **Sample position S2**
- **5 µL Droplet in PCR Tube**
- **In Science Commissioning**



Unfocused Beam

- **~2.5 x 2.5 mm FWHM**

100 µm pinhole scan with diamond detector

Sample Handling and Environment

- Capillary flow apparatus (4C water cooled) vertical or horizontal flow
- In-line incubator, fraction collector and multi-pump system for live cell experiments
- High-throughput samples RT to -30C
- Sample prep area at beamline with incubator, centrifuge, fluorimeter, UV/Vis

User Program

Science commissioning at XFP commenced in October 2016. Currently, commissioning of the capillary flow apparatus has been completed, and science commissioning with the high-throughput apparatus, in-vivo setup and time-resolved apparatus continue into the Summer 2017 cycle. General User operations are expected to phase in use of each apparatus starting with the capillary flow cell in Summer 2017. XFP will also accept rapid access proposals for several days each cycle.

The XFP beamline is available for users – please visit www.bnl.gov/ps/userguide to apply for beamtime.

Contact jbohon@bnl.gov to discuss your experiment.

Acknowledgements: The authors wish to acknowledge A. Hussein and D. Puleo for draft FE drawings and the support by the National Science Foundation Division of Biological Infrastructure under DBI-1228549 and DBI-1255340. The Case Center for Synchrotron Biosciences is supported by the National Institute of Biomedical Imaging and Bioengineering under P30-EB-009998. NSLS-II is supported by the U.S. Department of Energy under Contract No. DE-SC0012704.