X-ray Footprinting for *in Vitro* and *in Vivo* Studies of Biological Macromolecules (XFP) Beamline

17-BM

IRR Functional Description

PS-C-XFD-XFP-PLN-001

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1. INTRODUCTION

Primary Research Capabilities

The XFP beamline (X-ray Footprinting for *in Vitro* and *in Vivo* Structural Studies of Biological Macromolecules) is constructed as a partnership between Case Western Reserve University (with project funding through the National Science Foundation) and NSLS-II. This beamline is dedicated to solution state studies of biological macromolecular structure and dynamics, and will focus on the following research techniques:

1. *High-throughput Footprinting: For steady-state samples in simple buffers, experiments can be performed rapidly (initially 23 samples exposed in 5 minutes, expected to expand to 96 samples with future automation). This technique requires only 5 µL of sample per exposure, in micromolar to sub-micromolar concentrations, accommodating samples which are difficult to obtain in quantity. This experiment takes place using a 2.5 mm diameter sample at the second endstation area at XFP (defocused beam). The same experimental apparatus can be used to perform frozen-state x-ray footprinting (-30 C) to understand the role of water in cellular processes, including both impacts on both structure and function of macromolecules.*

2. *Steady-state Footprinting of Complex Samples: Capillary flow cells allow use of the fully focused beam (~100 – 700 µm capillary). This technique is used for membrane proteins, large macromolecular complexes, isolated sub-cellular organelles, and other research projects which require high flux density to overcome radical scavengers. This experiment takes place at the first endstation position, and requires approximately an order of magnitude more sample than the high-throughput experiments. This will also be expanded to include in-line FPLC experiments where unstable functional complexes and multiform samples are created and/or separated immediately before exposure to ensure study of the precise state of interest for the biological process under investigation.*

3. *Time-resolved X-ray Footprinting: Quench-flow mixing experiments allow studies of dynamics of biological molecules and their complexes in action on timescales from 100s of microseconds to minutes. This experiment takes advantage of a nearly-focused beam (~800 µm flow cell) at the first experimental endstation position, with similar sample requirements to the capillary flow cell.*

4. *In Vivo Footprinting:* P*robing molecular mechanisms of biological systems inside of living cells is possible with x-ray footprinting; these living cell systems contain significant hydroxyl radical scavengers and require the high flux density beam fully focused onto the capillary flow cell (first endstation position). For this experiment, live cells will be grown in an incubator proximal to the hutch, with a smaller in-line incubator on the beamline, a multi-pump system for controlled sample flow and sample collection performed via fraction collector. Precise measurement of the beam parameters and handling of the samples before and after the experiment at XFP enable this cutting-edge research.*

In conjunction with appropriate sample handling and ancillary laboratory facilities, these experimental areas cover the full range of the state-of-the-art in x-ray footprinting research. Changing endstation positions from the focused (first) endstation to the defocused positions further downstream in the hutch will require a vacuum change to extend the beam path to the sample without intervening air path to maintain flux on the sample. The XFP beamline construction project will initially complete the instrumentation to deliver beam to the sample positions, with continued support for development of endstation instrumentation from the National Institutes of Health through the Case Western Reserve University Center for Synchrotron Biosciences.

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2. BEAMLINE DESIGN AND COMPONENTS

2.1 BEAMLINE PERFORMANCE GOALS

Table 1 summarizes the designed performance of the XFP beamline. The beam size will be dictated by the needs of each individual experiment. The flux performance is estimated using ray-tracing simulations of the source and beamline optical components; the actual flux for each experiment will depend on the configuration of the optical components specific to the experiment.

Table 1. Designed performance of the XFP Beamline

Parameter	Specification/Description
Insertion Device	Three-pole wiggler
Operating Energy Range	Broadband 5-15 keV
Beam Size at Sample 1 position	Horizontal 0.5 mm, vertical variable from 0.1 to 0.8 mm
Beam Size at Sample 2 position	>2.5 mm diameter
Maximum Power Density at Sample 1 Position	>500 W/mm^2
Total Beam Flux	Up to 5 x 10^{16} photons/sec

2.2 BEAMLINE LAYOUT

The estimated performance listed above is based on the conceptual layout of the XFP beamline (Figure 1). The radiation source for the XFP beamline is a three-pole wiggler (3PW) installed at 17-BM. The only significant beamline optic, an upward-bounce toroidal focusing mirror, is located inside the front end at 14 m from the source for 1:1 focusing at the sample position at 28 m from the source. In order to direct the synchrotron beam into the hutch, the mirror must be maintained at a fixed 4.2 mrad angle. To achieve the required flexibility in beam size, the vertical dimension can be changed via adjustment of the meridional bend radius of the toroidal focusing mirror and the horizontal dimension via positioning of the sample along the beam path.

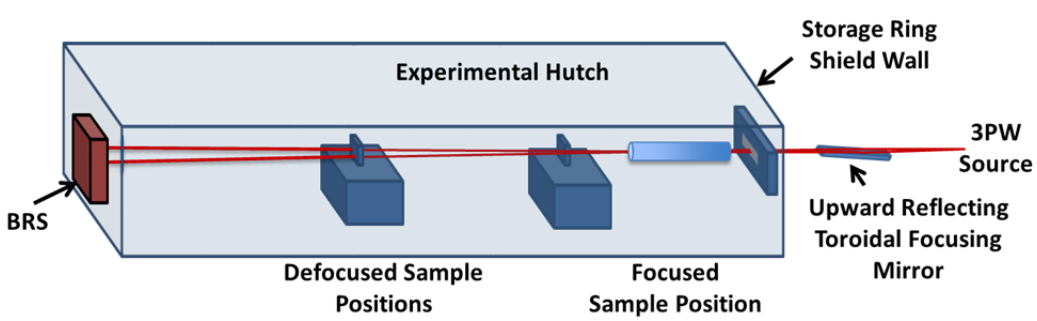


Figure 1: Schematic layout of the XFP beamline. A toroidal focusing mirror, located inside the front end at 14 m from the 3PW source provides 1:1 focusing of the beam onto the sample position at 28 m from the source. The FOE (experimental hutch) is located immediately downstream of the shield wall. Multiple sample locations are shown for different sample experimental needs.

The 3PW source characteristics are summarized in Table 2. The source will provide broadband radiation from <1 to 25 keV, filtered via beamline optics to the desired 5-15 keV (Figure 2). The beamline is designed to include two windows, a beryllium window upstream of the toroidal mirror, and an instrumented diamond exit window at the end of the beamline just prior to the sample. White beam slits are located inside the front end and pink beam slits are located inside the FOE for beam definition as desired.

Table 2: Source Parameters for the NSLS-II Three-Pole Wiggler. [Source: "NSLS-II Source Properties and Floor Layout," April 2010]

Peak Field	1.14 T
Length	0.25 m
Number of Periods	0.5
Magnetic Gap	28 mm
Critical Energy	6.8 keV
On-axis Power Density	260 W/mrad^2
Maximum Total Power	320 W

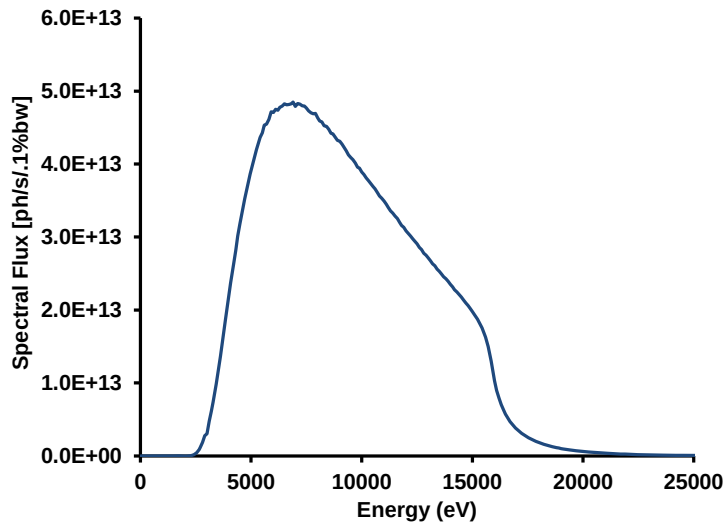


Figure 2: Spectral flux at XFP from 3 horizontal and 0.33 vertical mrad of 3PW radiation, filtered through 254 µm of Be and 75 µm of diamond at a mirror angle of 4.2 mrad (Rh-coating).

The photon energy range at XFP is determined by a combination of the reflectivity of the mirror in the front end (high energy cut-off at ~15 keV) and the set of filters (including windows consistently in the beamline and removable aluminium plates of differing thicknesses chosen based on the experimental needs) which will determine the low energy cut-off. The flux obtained, beam position, and beam shape/size will be measured via a transmission-mode diamond imaging detector (expected to be integrated into the beamline exit window in the future).

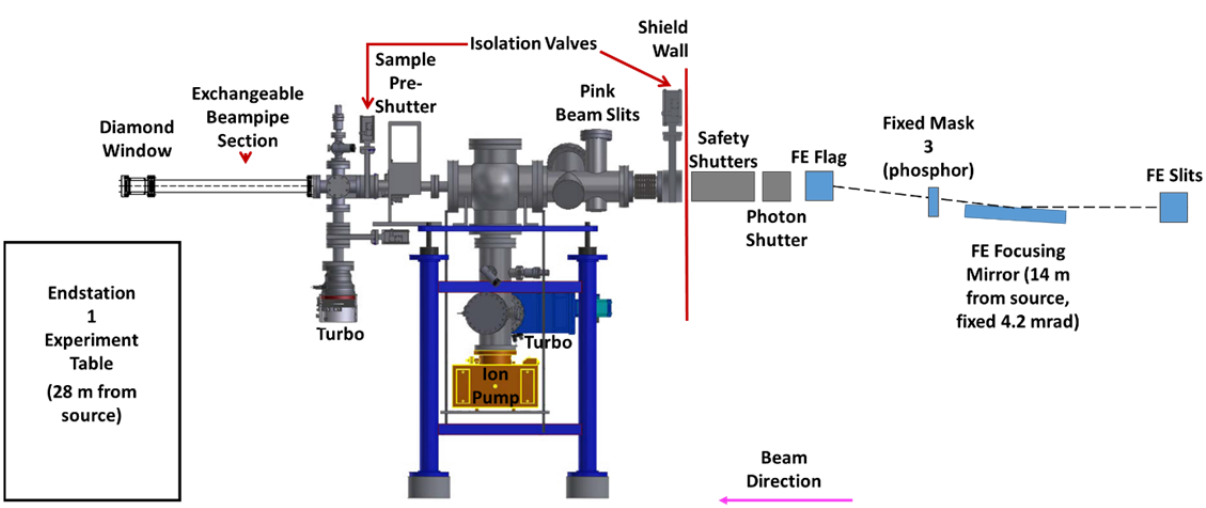


Figure 3: Overview of beamline components at XFP

The majority of the XFP beamline is located inside of the front end; an overview of the major beamline components is provided in Figure 3 and a detailed layout of the XFP Front End is provided in the appendix. Front end slits allow definition of the beam incident on the focusing mirror; these slits are also instrumented to function as a monitor of the white beam position. Two additional beam observation points are installed in the front end; the mask immediately downstream of the focusing mirror is fitted with a screen to indicate misalignment, and a retractable flag is also available just upstream of the photon shutter. Downstream of the shield wall, the pink beam slits enable beam definition on the sample, and a pre-shutter is installed for sample protection prior to exposure. Once samples are exposed, the samples are collected for analysis via mass spectrometry or gel electrophoresis (dependent on sample type); no detector is required at the beamline for data collection other than logging beam parameters during an experiment.

3. BEAMLINE SAFETY

3.1 RADIATION SHIELDING

The design of all radiation shielding (hutches and radiation safety components) follows guidelines to reduce radiation levels external to the beamline enclosure during normal operation to < 0.05 mrem/hr and as low as reasonably achievable. The shielding wall thicknesses follow released shielding guidelines [source: W.-K Lee et.al., Guidelines for the NSLS-II Beamline Shielding Design, (LT-C-ESH-STD-001)].

Hutch A (FOE, white beam hutch):

- Lateral wall: 18 mm lead
- Downstream wall: 50 mm lead
- Roof: 10 mm lead

The XFP first optical enclosure (FOE) 17-BM-A will also serve as the experimental hutch.

3.2 RADIATION SAFETY COMPONENTS AND CONFIGURATION CONTROL

These are the components required to contain the synchrotron radiation from the 3PW source and the gas Bremsstrahlung radiation from the storage ring. These components at 17-BM include the 5 roof labyrinths, the PPS labyrinth inside the 17-BM-A hutch, the guillotine, and the Bremsstrahlung beam stop (BRS) downstream of the hutch (listed in the beamline radiation safety component checklist). The guillotine at the downstream end of the hutch is fully closed and covered with a stainless steel sheet for heat spread in the case of incident pink beam on the rear hutch wall. The BRS is permanently installed immediately downstream of the 17-BM-A hutch and is required for top-off operation at the beamline (PS-C-ASD-TOS-RPT-004). All radiation safety components are under

configuration control, following the NSLS-II Radiation Safety Component Configuration Management procedure (PS-C-ASD-PRC-055).

3.3 BREMSSTRAHLUNG RADIATION MANAGEMENT

Due to the geometry arising from having a focusing mirror inside of the front end, primary Bremsstrahlung radiation is contained within the front end and does not enter the 17-BM-A hutch. Secondary (scattered) Bremsstrahlung radiation can arise from scattering of primary Bremsstrahlung radiation off the focusing mirror and off of Fixed Mask 4 in the front end. Based on NSLS-II guidelines, an additional Bremsstrahlung radiation shield (BRS in Figure1) is installed. FLUKA simulations and a number of design iterations were carried out to verify that the dose rate outside the 17-BM-A hutch is below the limit of 0.05 mrem/hr at 500 mA operational beam current.

3.4 OXYGEN SENSOR

To mitigate the potential production of ozone at the beamline exit window (which could damage the window), a low but continuous flow of nitrogen gas will be used at the window position in the 17-BM-A hutch. Although the flow to be used on the window will not create an oxygen deficiency hazard, potential accidental rupture of gas lines in the hutch feeding this flow could pose such a hazard. This is mitigated by the installation of an oxygen sensor inside the hutch. The system has been certified in accordance with procedure PS-C-XFD-PRC-005, Beamline Enclosures ODH Monitoring and Alarm System Certification and Inspection.

3.5 PERSONNEL PROTECTION SYSTEM (PPS)

The Personnel Protection System (PPS) controls access to the hutch through the interlock system and beamline-specific search and secure procedure to ensure personnel safety during normal operation of the beamline. The hutch is equipped with a PPS-interlocked user labyrinth to facilitate temporary equipment access during user experiments.

3.6 HAZARD IDENTIFICATION AND MITIGATION

The XFP beamline is similar to other beamlines already in operation at NSLS-II with respect to any hazards that might be encountered. A USI evaluation has been conducted and it was determined that the anticipated activities at the beamline do not violate the existing SAD and ASE. All relevant NSLS-II procedures and safety practices are followed during the design and construction of the beamline to mitigate the hazards identified in these documents.

4. INSTRUMENT READINESS

4.1 SURVEY AND ALIGNMENT

The beamline components are installed according to the specifications and the respective final designs. Installation of the components are verified and documented by the NSLS-II Survey group working closely with beamline staff.

4.2 UTILITIES

The following services/capabilities are deployed at the beamline:

- Electrical power distribution: to all racks, electrical power outlets, light fixtures, fans, etc. in the hutch and along the beamline
- Distribution of deionized water to the hutch to beamline components requiring water cooling
- Distribution of process chilled water to the hutch and water-cooled racks
- Compressed air: for pneumatic valves
- Dry nitrogen gas
- Network connectivity
- Cabling and piping support structures, for all utilities including EPS and PPS.

4.3 VACUUM SYSTEM AND PRESSURE SAFETY

The vacuum pressure for the beamline (downstream of GV2) is expected to be $2x10^{-6}$ Torr or better. The beamline vacuum is separated from the accelerator vacuum by a beryllium window located in the front end. The beamline vacuum section, when all valves are open, is shared with the vacuum for the focusing mirror chamber. An ion pump is installed on the photon delivery system and several additional ion pumps are located inside the front end downstream of the beryllium window to provide differential pumping to preferably maintain the mirror chamber at a vacuum pressure of better than 10^{-8} Torr.

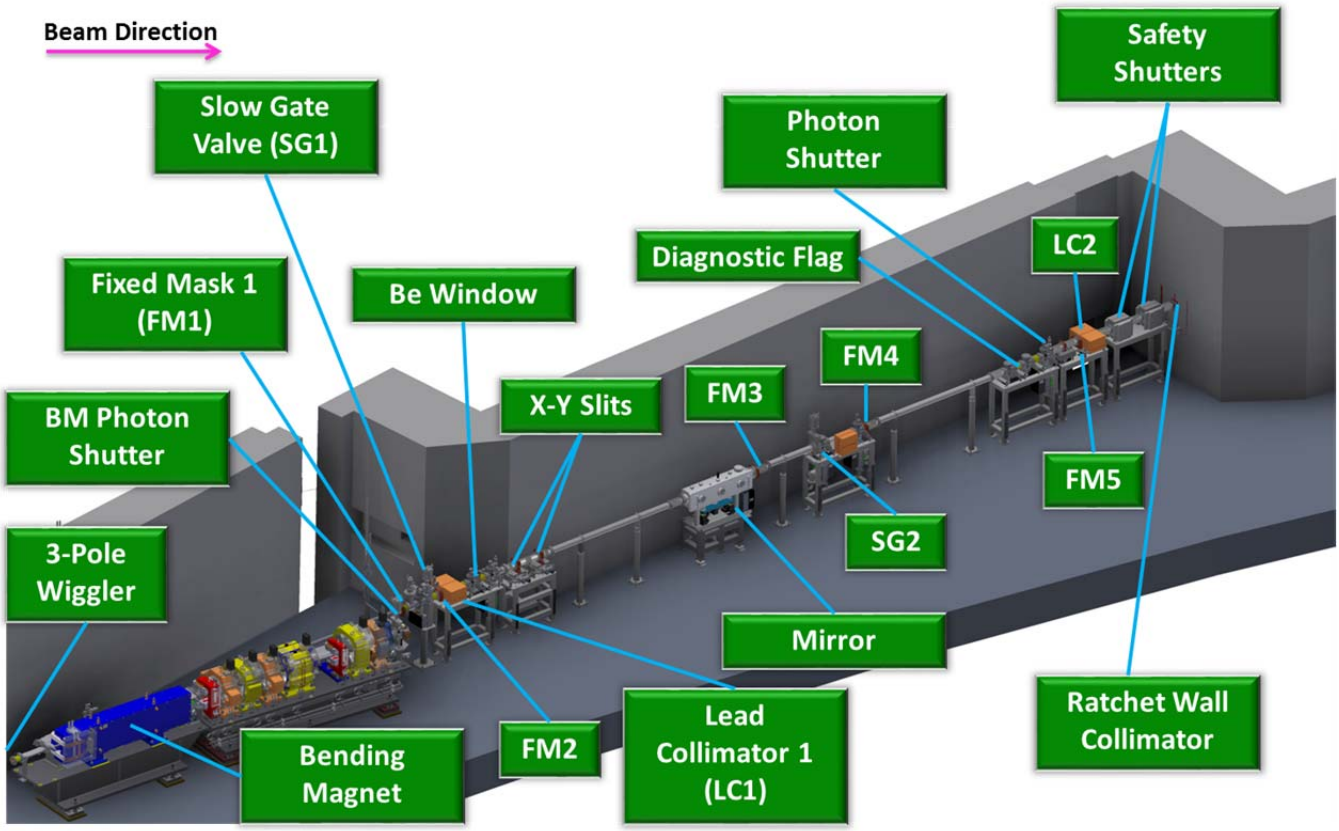
When venting vessels to dry nitrogen gas, a pressure relief valve with a very low relieving pressure (1/3 psi) will be used to prevent any internal overpressure condition. Pressure tests have been performed on the final diamond vacuum windows (interchangeable) used at the beamline that terminate the flight path in the experimental station.

4.4 CONTROLS

All motorized components have been tested and documented in the appropriate travelers. Controls System Studio (CSS) screens have been prepared to access the motors on the components.

4.5 EQUIPMENT PROTECTION SYSTEMS (EPS)

The Equipment Protection System at the XFP beamline performs the following functions:

- Vacuum pressure monitoring and interlock for required vacuum sections of the beamline
- Temperature monitoring for all non-safety related components, including the white beam mirror system and water-cooled electronic racks
- Water flow monitoring, leak detection and interlock for the cooling of the pink beam slits and sample shutter
- EPICS interface for components that require I/Os installed on the EPS PLC, including the readout of thermocouples, control of venting and evacuation valves in the experimental station, and actuation of the sample shutter and front end flag

Appendix 1: Layout of the 17-BM Front End