Microfabrication of Fixed Length Sample Holders for Cryogenic Small Angle X-Ray Scattering

CNF Project Number: 2157-12

Principal Investigator(s): Robert Thorne

User(s): David Moreau

Affiliation(s): Cornell Laboratory of Atomic and Solid State Physics, Cornell University

Primary Source(s) of Research Funding: National Institutes of Health

Contact: ret6@cornell.edu, dwm265@cornell.edu

Primary CNF Tools Used: Heidelberg mask writer - DWL2000, SÜSS MA6-BA6 contact aligner, Oxford 80, Objet30 Pro 3D printer, VersaLaser engraver/cutter tool, YES polyimide curing oven, potassium hydroxide wet etch station

Abstract:

Small-angle X-ray scattering (SAXS) is a key tool for probing the structure and function of proteins, nucleic acids, and macromolecular complexes. Most synchrotron sources have dedicated BioSAXS beam lines, but efforts to improve their throughput have not kept pace with user demand. Large sample volumes and low duty cycles are critical bottlenecks in the expansion of BioSAXS. Cryogenic sample freezing overcame these bottlenecks in an analogous X-ray technique, macromolecular crystallography. Cryocooling significantly reduces the effects of X-ray radiation damage, reducing the necessary sample volume to collect adequate amounts of data, and eases the sample handling of sensitive or unstable samples. Likewise, CryoSAXS should require much smaller sample volumes per measurement, allow sample preparation in the home lab immediately after purification, easy sample storage and shipping, and automated high-throughput data collection. This will enable dramatically more efficient use of both biomolecules and synchrotron beam time, and significantly expand the potential scope of BioSAXS studies.

Summary of Research:

We envision CryoSAXS as a routine method analogous to cryocooling in macromolecular crystallography (MX). The reduction in radiation damage at T = 100 K significantly reduces the amount of protein required per measurement and sample holders compatible with standard macromolecular cryocrystallography (MX) infrastructure could be transformative step in increasing the throughput and potential of BioSAXS. CryoSAXS could be especially useful for high-throughput parameter and ligand interaction screening and for study of difficult to produce proteins or complexes, applications in which BioSAXS may have the greatest impact on human health.

Despite the demonstrations of its potential [1,2], the lack of a robust experimental platform has prevented CryoSAXS from becoming a routine experimental technique. The need to subtract a highly matched background scattering pattern from the macromolecule's scatter and the difficulty in vitrifying bulk-like solutions have posed serious technical challenges for the development of sample holders adequate for routine use. Shown in Figure 1 is a new generation of CryoSAXS devices we recently developed using microfabrication techniques at the Cornell NanoScale Science and Technology Facility (CNF).

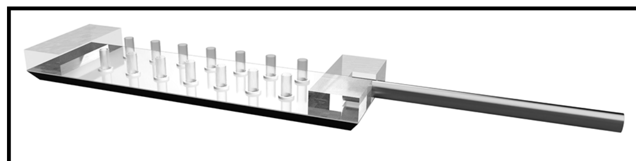

Figure 1: Computer generated image of CryoSAXS sample devices. The sample is held in place between two silicon nitride X-ray windows by Kapton tube. The X-ray passes axially through the tubing. Multiple sample cells are present in a single device in two rows. One row contains solutions with a macromolecule and the other is analogous solutions without the macromolecule to be used for background subtraction.

These devices constrain the sample held between two silicon nitride windows at a 1 mm fixed pathlength. Double-sided polished wafers coated in 500 nm of low-pressure chemical vapor deposited silicon nitride were procured from Silicon Valley Microelectronics. One side of the wafer was patterned with photoresist then dry etched to remove the nitride for a later potassium hydroxide (KOH) wet etch. SU-8 was deposited and patterned on the other side to serve as a hydrophobic layer to aid in the pinning of the sample to the X-ray windows. A KOH wet etch then formed the X-ray windows and diced the wafer. Spacers were laser cut

from 1 mm thick quartz glass sheets then glued to one portion of the wafer pieces. Using laser cut jigs, 1 mm long Kapton tubing was cut and glued to the devices. The devices are filled from the open end of the tube, then a second wafer piece is affixed to the top of the device to seal the sample. The samples are then cryogenically frozen by plunging into liquid nitrogen (LN_2), stored in LN_2 and later mounted in an X-ray beam while being kept at T = 100 K by a cold gas stream.

X-ray data collection was performed at Cornell High Energy Synchrotron Source (CHESS) beamline ID7A1 for the protein apoferritin using 45% w/w propylene glycol as a cryoprotectant. Figure 2 shows results from apoferritin at three different protein concentrations. Figure 2a shows the background subtracted X-ray diffraction intensity. The inset shows this data in a Gunier plot. Deviations from linearity at low scattering angles are indicative poor data quality, including incorrect background subtraction at low scattering angles. The normalized Kratky plot in Figure 2b. This should go to zero at higher scattering angles for globular proteins such as apoferritin. Deviations indicate incorrect background subtraction at high scattering angles.

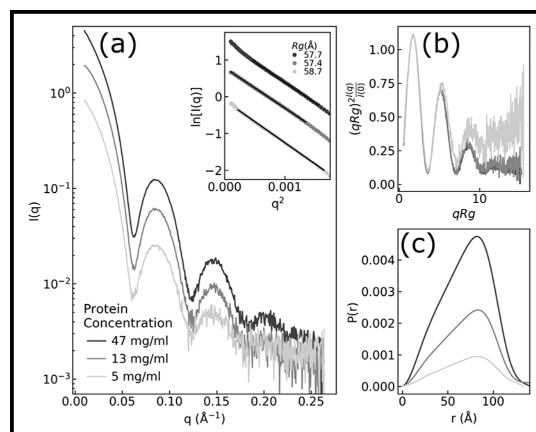

Figure 2: Background subtracted X-ray diffraction intensity from apoferritin at three different concentrations.

Conclusions and Future Steps:

The development and subsequent adoption of cryogenic methods for BioSAXS could significantly increase sample throughput and reduce sample consumption, enabling SAXS to extend to a larger user base. It could open up the possibility of new science through high-throughput screening of ligands or other therapeutics. CryoSAXS's potential has been known for a while, but critical technical challenges have prevented the widespread adoption of the method. A well-matched background for subtraction is necessary for SAXS and the cryocooling step tends to introduce significant variability to the background. X-ray scatter from ice present in the sample quickly overwhelms the scatter from the protein, as does parasitic scatter from a sample that has cracked under the stress induced by cryocooling. This new generation of devices is designed to maximize the sample's cooling rates and minimize internal stress. Future steps involve the refinement of this technique through iterative design.

References:

[1] Meisburger, S. P., et al., (2013) Biophys. J., 104, 227-236.

[2] Hopkins, J. B., et al., (2015) J. Appl. Cryst. 48, 227-237.