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

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Principal Investigator(s): Robert Thorne

User(s): David Moreau, Jonathan Clinger, Liam Barnes

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

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

Contact(s): ret6@cornell.edu, dwm265@cornell.edu

Website: https://www.lassp.cornell.edu/Thorne/

Primary CNF Tools Used: Heidelberg mask writer - DWL2000, SÜSS MA6-BA6 contact aligner, Oxford 81/82, VersaLaser engraver/cutter tool, YES polyimide curing oven, SUEX laminator, Hamatech hot piranha, LPCVD CMOS Nitride - E4, Class II resist room

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 procedure 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, the study of difficult to produce proteins or complexes, and extremely radiation sensitive targets, 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).

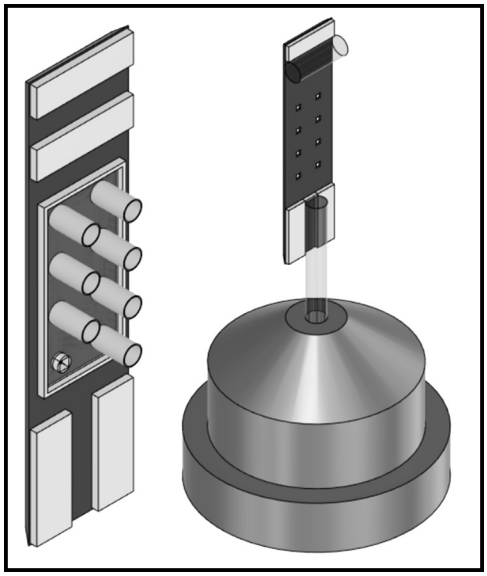

Figure 1: Images of CryoSAXS sample devices. The sample is held in place between two silicon nitride X-ray windows by Kapton® tube. The X-ray passes axially through the tubing. Multiple sample cells are present in a single device in two rows. One row contains solutions with a macromolecule and the other 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 deposition silicon nitride using

the LPCVD CMOS nitride furnace (E4). Using the MA6-BA6 contact aligner, one side of the wafer was patterned with photoresist. The nitride was then dry etched with the Oxford 81 plasma etcher for a later potassium hydroxide (KOH) wet etch. SUEX was laminated onto the other side and patterned with backside alignment using the MA6-BA6 contact aligner. The SUEX features serve as guides for alignment and to help position tubes. A KOH wet etch then formed the X-ray windows and diced the wafer. The VersaLaser was used to cut spacers from 1 mm diameter quartz glass rods and affixed to one wafer pieces. Using jigs for cutting and alignment, 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 in a cold nitrogen gas stream at T = 100 K for data collection.

X-ray data collection was performed at Cornell High Energy Synchrotron Source (CHESS) beamline ID7A and NSLS-II beamline 16ID for the protein apoferritin using 35% w/w propylene glycol as a cryoprotectant. Figure 2 shows results from apoferritin at several protein concentrations.

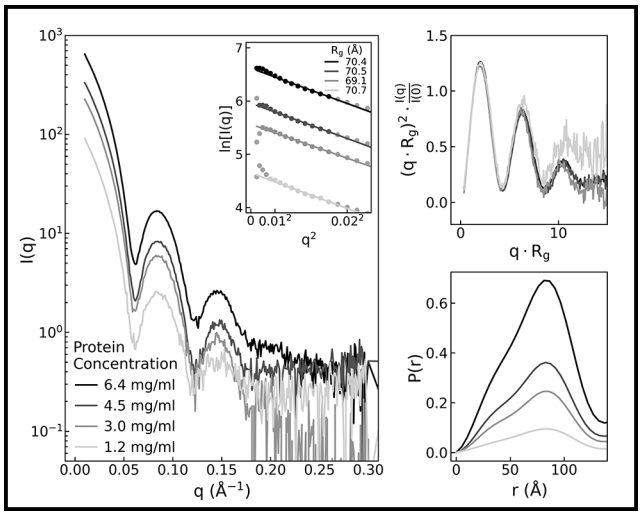

Figure 2: Background subtracted X-ray diffraction intensity from apoferritin at different concentrations.

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.