Microfabrication of Sample Holders for Cryogenic Small Angle X-Ray Scattering and Flow Cells for Fluorescence Measurements of Ligand Diffusion in Protein Crystals

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Primary CNF Tools Used: Heidelberg Mask Writer - DWL2000, SÜSS MA6-BA6 Contact Aligner, Oxford 81 / 82 PlasmaLab, VersaLaser Engraver/Cutter, YES Polyimide Curing Oven, SUEX Laminator, Harrick Plasma Generator, Hamatech Wafer Processor, LPCVD CMOS Nitride - E4, Filmetrics Reflectometer, Class II Resist Room

Abstract:

We use microfabrication in our development of methods for probing the structure and dynamics of proteins and other biomolecules. We have fabricated sample cell arrays for high-throughput small-angle X-ray scattering of biomolecules that allow samples to be "immortalized" by cooling to cryogenic temperatures. We are also fabricating crystal trap flow cells for quantitative fluorescence measurements of diffusion coefficients of small molecules inside protein crystals, critical parameters for design and interpretation of time-resolved X-ray crystallography experiments probing enzyme function.

Summary of Research:

CryoSAXS Sample Holders. Small-angle X-ray scattering on biomolecules in solution at room temperature is a workhorse tool for determining biomolecular size, shape, and changes in these due to interactions with other molecules or environmental changes. Our goal is to enable SAXS measurements on samples cooled to (and stored and shipped at) cryogenic temperature. This will allow sample preparation in the home lab as soon as protein is purified, long-term storage, and mail-in, remote data collection at synchrotrons, while dramatically reducing radiation damage and required sample volumes [1].

We have developed and have been evaluating and evolving sample cell arrays for cryoSAXS, shown in Figure 1. The cell arrays consist of pairs of 300 μm thick double-side polished Si pieces, coated on one side with 500 nm of silicon nitride, and KOH-etched to form X-ray transparent nitride windows. Before KOH etching, SUEX sheet is bonded to the nitride-coated side and patterned to form alignment and sealing rings. Thin-wall polyimide tubes are bonded to the rings on one piece, and quartz spacers bonded to fix the X-ray path length through the tubes between windows. X-ray

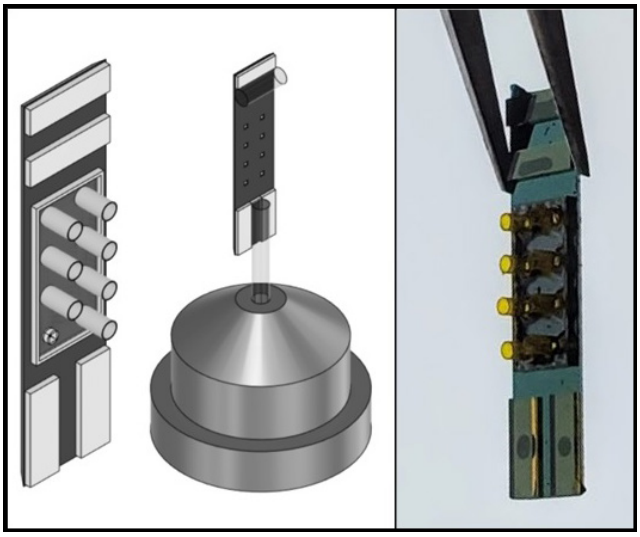

Figure 1: CAD image and photograph of cryoSAXS sample cell arrays. Each sample is held within a polyimide tube between two silicon nitride X-ray windows. The X-ray beam passes axially through the tube. Measurements from two cells in the array, one containing protein+buffer solution and one protein-free buffer solution, must be subtracted to obtain information about the biomolecular structure. As the subtracted diffraction may be 10^{-3} of the total, the cells must be identical.

experiments at CHESS and NSLS-II show that these cells allow rapid cooling of biomolecular samples without ice nucleation and without cracking seen due to differential contraction of sample and cell components (seen in previous cells [2]). However, experiments in the last 18 months identified issues with parasitic upstream X-ray scatter and how this scatter interacts with the sample cells that lead to unacceptable irreproducibility. We are currently addressing this issue, which we believe to be the final major obstacle to obtaining high-quality cryoSAXS data, through changes in cell design and in the experimental configuration at the X-ray beamline. We are also pursuing a simplified, all-microfabricated cell array design in which the polyimide tubes are replaced with high aspect ratio SUEX tubes.

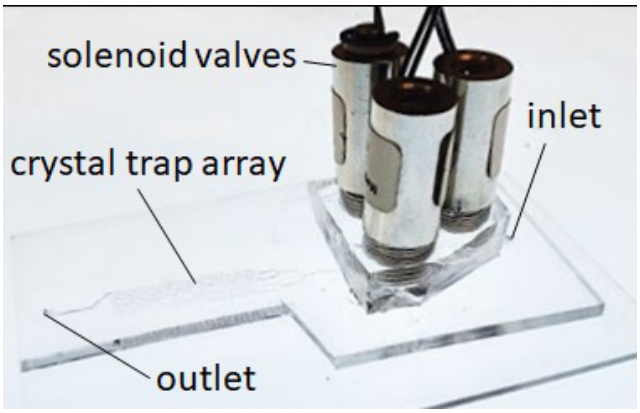

Figure 2: Microcrystal trap array formed by molded PDMS on a glass slide, for measurement of fluorophore diffusion in protein crystals. Solenoid valves control flow of protein crystal-and fluorophore-containing solutions.

Crystal Trap Array Flow Cells. Studying enzymes in action with atomic resolution has been a holy grail in structural enzymology. The most promising methods involve mixing enzyme microcrystals with reactant solution, allowing the reactant to diffuse into the crystal, and then measuring X-ray diffraction at different time delays after mixing [3]. Experiments so far have assumed that diffusion coefficients of reactants in protein crystals are the same as in bulk solution, but theoretical analysis of related problems suggest they may be as much as 10 to 100 times smaller, with major consequences for interpretation of time resolved diffraction experiments.

We are attempting to measure diffusion coefficients of small molecules in protein crystals using either fluorophores or molecules with distinctive intrinsic fluorescence / absorption. To do so, we have fabricated a microcrystal trap array (Figure 2), based on the design and fabrication protocol described in [4]. The traps are loaded by flowing a microcrystal-containing solution through the array. Then a solution containing the small molecule fluorophore is sent through the array, and fluorescence monitored both inside the crystal (using laser scanned microscopy) and in the surrounding solution. The trap array is fabricated from PDMS, and flows are controlled by solenoids.

A master is fabricated by laminating a 100 μm thick SUEX sheet to a silicon wafer, which is then exposed using a mask to define the features of the trap array, and developed. PDMS is poured onto the master with a target thickness of 2 mm, cured, removed, and then bonded to a glass slide. A second, 5 mm thick PDMS layer is then fabricated with screws, centered over the location of input flow channels of the first layer, cast within it. These screws are removed, the second layer bonded to the first, and solenoid valves threaded into the holes. When activated, each solenoid presses down on the first layer of PDMS and collapses the inlet channel over which it is situated.

We successfully tested a valve-free version of the trap array, obtaining fluorescence data from 50 μm crystals and credible diffusion coefficients. The valved version will simplify data collection and improve time resolution.

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.

[3] Clinger, J. A. et al., (2021). IUCrJ 8, 784-792 (2021).

[4] Lyubimov, A. Y. et al., Acta Cryst. D 71, 928-940 (2015).