An *in vacuo* Microfluidic Mixer for Biological X-Ray Solution Scattering

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Primary CNF Tools Used: Heidelberg mask writer DWL2000, SUEX laminator, ABM contact aligner

Abstract:

Time-resolved small angle x-ray solution scattering (TR-SAXS) remains a challenging, but increasingly important experiment for structural biologists. Such experiments can tell researchers about multi-step conformational changes biological molecules undergo as part of their function. The continuous-flow mixing that utilizes the chaotic laminar flow regime of fluids provides a means of reaching single millisecond timescales with disposable plastic microchip construction and minimal sample consumption. Photolithographically fabricated mixing chips have been tested *in vacuo* to reduce parasitic x-ray scatter.

Summary of Research:

Small-angle x-ray solution scattering (SAXS) is a widely used technique in structural biology for gaining information about the behavior of molecules in solution. Time-dependent SAXS studies have been conducted for a number of years, with significant advances being made in design, sample consumption, and instrumentation [1]. But adoption of the method by non-specialists has been slow in coming, and the method remains a challenging experiment. To address the need for easier, more practical experiments, we introduced a system based on the principle of chaotic advection [2].

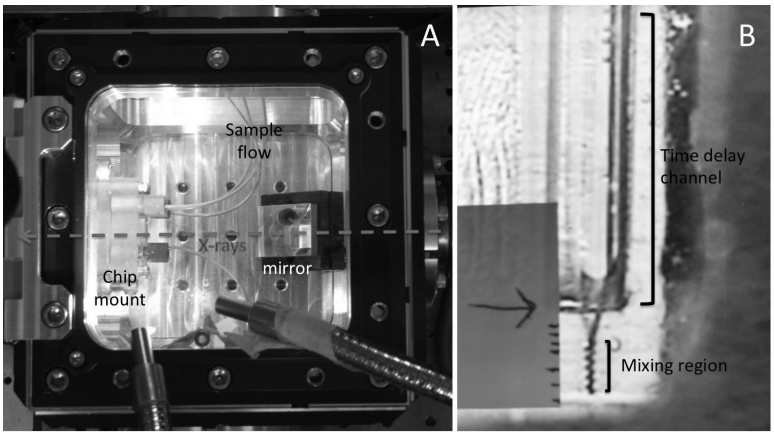

Figure 1: Microfluidic mixing chip for SAXS mounted in vacuo at CHESS beamline G1. A: X-rays (red) pass through cubic vacuum enclosure over viewing mirror and through microfluidic cell. B: Mixing region and time-delay channel of chip are visible in camera snapshot.

Our previously designed mixing chip, fabricated at CNF, is a composite of SUEX (DJ Microlaminates Sudbury, MA), polymethylmethacrylate (PMMA) and polyimide layers driven by a commercial piezo-controlled pressure system (ELVESYS, Paris, France).

Thin polyimide film (7 μm) serves as low-scatter windows for x-ray transmission. To eliminate x-ray scattering due to air and vacuum windows, we have designed an enclosed sample environment that allows mixing chips to operate in vacuum (Figure 1A). Polyether ether ketone (PEEK) sample feed tubing enters the vacuum through KF 50 blanks into the cubic sample enclosure (Ideal Vacuum, Albuquerque, NM) and connects to the microfluidic chip via flangeless fittings.

Positioning of the x-ray beam in the chip channel is accomplished by scanning the cube assembly in the x-ray beam and monitoring transmitted intensity. The chip can be viewed with a remote camera via a diagonal mirror just below the path of the x-ray beam (Figure 1A, right). The mixing and time-delay channels are visible in the camera image (Figure 1B). X-ray tests using a novel compound refractive optic for focusing down to 30 μm showed relatively low parasitic scatter though the time-delay channel.

References:

[1] Graceffa, R., et al., Sub-millisecond time-resolved SAXS using a continuous-flow mixer and x-ray microbeam. Journal of Synchrotron Radiation, 2013. 20: p. 820-825.

[2] Kane, et al. Microfluidic Mixers for the Investigation of Rapid Protein Folding Kinetics Using Synchrotron Radiation Circular Dichroism Spectroscopy. Anal. Chem. 2008, 80, 9534-9541.