Design, Fabrication and Testing of Dual Input, Mixture Inducing BioSAXS Flow Cell

Abstract

Advances over the past several decades in microfluidic devices and associated fabrication techniques have enabled considerable interest in the structural biology community. Current boundaries of these technologies are being probed to the nanoscale with complex devices that allow for rapid mixing and visualization of fluids. Common applications exist in fluid dynamics in both laminar and turbulent flow regimes. Another practical application is to combine streams of protein and lipids, and directly afterwards (within milliseconds) apply an imaging modality to the mixed stream. There has been much focus on expanding our capabilities that a single measurement technique can offer. BioSAXS is emerging as a popular complimentary technique to cryo-EM and is well suited to study protein dynamics. This project aims to develop the necessary technology to study the full range of protein dynamics at small time scales (e.g., times after mixing) in BioSAXS. BioSAXS utilizes two laser beams, collinear and counter-propagating, for simultaneous detection of the mixture and the signal. In this work it is shown that it is possible to image more realistic natural structures, and that BioSAXS has a high level of versatility for analyzing the mixtures in terms of the mixing event (input/output). At the same time, users will have the ability to increase their experimental throughput. 1000 measurements per day are possible to image enough protein for analysis. Grimes et al., used BioSAXS to image protein diffusion (NIH, 2008:170). The BioSAXS facility at Cornell Nanoscale Facility is shown above.

BioSAXS vs Crystallography

- Small volume, which results in a Reynolds number (Re) of 100 or less.
- Stage representation
- Isothermal conditions

SAXS/WAXS

- Losses in sample collection
- Inherent barrier to study large scale structures
- Deformation of proteins and other large molecules

Current BioSAXS Flow Cells

Once the layers have been set out with the Varian laser, they are brought back to the G3 chemistry flow to be assembled into flow cells with the following protocol:

1. Layers are cleansed with isopropyl alcohol.
2. The first layers to be bonded are 1 and 2, this is because the polystyrene (Varian transparent window) is to be placed onto the sides that outline the flow chamber (layers 3 and 4) and need several minutes to bond securely. These layers are 1.5 mm thick and require 9 minutes of chromat layer vapor exposure to bond well with the polystyrene.
3. When selecting layers 2 and 4, layers 2 and 4 are placed into the vacuum container, and are Varian laser exposed by 19 minutes so that layers 2 and 4 are bonded and compressed in a vise to ensure tight binding.
4. Clear liquid, and it must be exposed to layers for 10 minutes before being added onto the layers and sealed inside into the vise.
5. Ideally compressed glue should be allowed to flow through the cells while they are bonding to avoid residual liquid finding the Varian windows, but this was not a particularly promising approach because these cells were fabricated primarily for testing fluid flow and mixing potential. Future work will be fabricated with sufficient film.

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