Microfluidic Protein Dialysis Device for X-Ray Scattering

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Abstract and Introduction:

The purpose of the microfluidic protein dialysis device is to create an in situ protein enrichment device for small-angle x-ray scattering (BioSAXS) on a synchrotron beamline, allowing a protein’s structure to be determined. The device allows users to create concentration series necessary for BioSAXS starting with a diluted sample, rather than diluting a pre-concentrated sample. This avoids potential irreversible aggregation of protein molecules in solution.

Most of the research was conducted using a five-layer device, consisting of two layers of polymethyl methacrylate (PMMA), two layers of double-sided medical adhesive tape, and a semi-permeable cellulose dialysis membrane. The CNF’s VersaLaser 3.50 was used to carve channels into each tape layer, to allow a lysozyme protein solution, a commonly used standard for testing structural biology, to flow through one side, while a polyethylene glycol (PEG) solution flowed through the opposite channel.

By the process of osmosis, water moved from the protein solution across the semipermeable membrane into the PEG solution, resulting in a concentrated protein solution. The five-layer device was tested using a spectrophotometer to determine the absorbance of protein solutions at various protein solution and PEG flow rates. The results obtained thus far indicate the protein was being concentrated.

Preliminary flow tests for a seven layer chip containing two dialysis membranes, and results of a Polydimethylsiloxane (PDMS) device, inspired by Chijung et al’s article [1], are also presented.

Experimental Procedure:

The construction of the five-layer protein dialysis device, shown in Figure 1, began by using the VersaLaser to carve channels 1.00 mm wide and 0.05 mm deep into the tape, and 1.6 mm wide holes into the PMMA. The pieces were prepared, placed on top of each other, and aligned. Air bubbles were removed by tapping the device with a scissor handle. Teflon® tubes, used to allow fluid in and out of the device, were placed in the PMMA holes and adhered with epoxy and super glue. The device was tested with different

![Figure 1: Side-view schematic of five-layer device.](image1)

![Figure 2: PDMS device creation: (a) Resist on wafer, (b) Etch, (c) Resist Removal, (d) PDMS on wafer, (e) Final device.](image2)
covering the entire tape area with dialysis membrane, rather than just the channel area, and by placing epoxy around the sides of the device. To allow for more concentration, new devices can be designed to have longer channels, with a port for the x-ray. Also, the device should be tested in the synchrotron with an x-ray beam.

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References:

Graph 1: Change in PEG flow rate with 0.1 mg/ml protein

Graph 2: Change in PEG flow rate with 1 mg/ml protein

Graph 1, top: Changes in PEG flow rates, from 0.25 µl/min to 3 µl/min, with 0.1 mg/ml protein solution.
Graph 2, bottom: Changes in PEG flow rates, from 0.25 µl/min to 3 µl/min, with 1.0 mg/ml protein solution.