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.

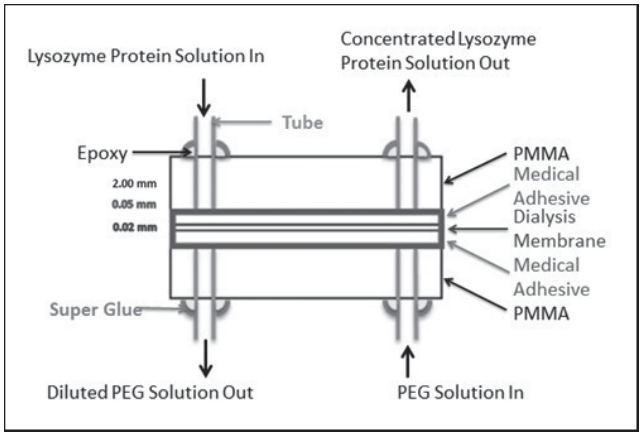

Figure 1: Side-view schematic of five-layer device.

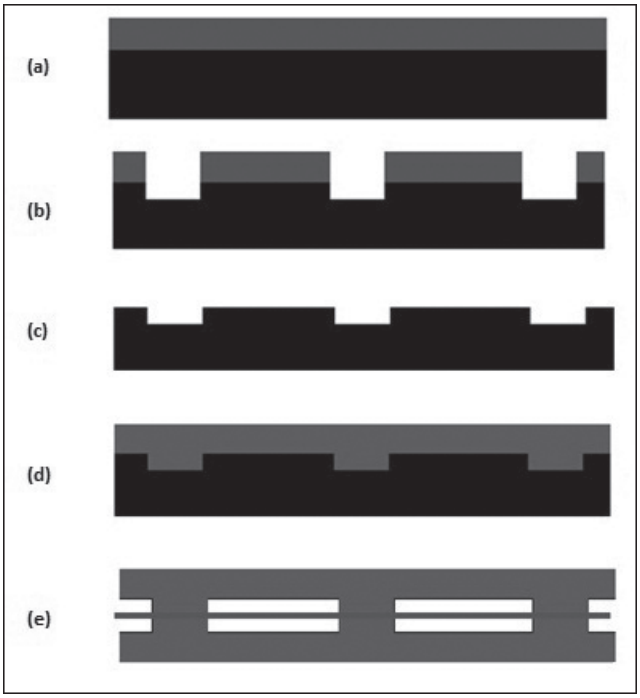

Figure 2: PDMS device creation: (a) Resist on wafer, (b) Etch, (c) Resist Removal, (d) PDMS on wafer, (e) Final device.

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

protein and PEG flow rates, controlled by pumps, for 0.1 mg/ml and 1.0 mg/ml starting protein solutions. The absorbance and concentration values were obtained by UV absorption spectroscopy at 280 nm.

The seven-layer device had one tape, and dialysis membrane, layer in addition to the five-layer device.

The construction of the PDMS device, shown in Figure 2, began by spinning negative resist, nLOF2020, onto a silicon wafer, undergoing a soft bake, exposing, undergoing a post exposure bake, developing, etching with the Unaxis 770, and removing resist with a hot bath. PDMS was then poured over the wafer, with protection to easily remove the PDMS. The chip was then assembled by placing the PDMS and a dialysis membrane in a Harrick Plasma Cleaner and layering as seen in Figure 2.

Results and Conclusions:

As seen in Graphs 1 and 2, the five-layer device was able to concentrate a protein sample, sometimes by two-fold, and provide promising results. Graph 1 results were collected using UV absorption spectroscopy. The absorbances were converted to concentrations using the Beer-Lambert Law, $A = EC\ell$. Graph 2 shows much fluctuation, which could be the result of human or machine error. The data was collected using a NanoVue spectrometer to minimize sample evaporation. Although there was concentration, there is no clear relation between flow rates and concentration.

While long-term adhesion of the dialysis membrane to the tape and PDMS remains an issue, preliminary tests with 0.1 mg/ml and 1.0 mg/ml lysozyme solutions indicate that concentration is occurring on a reasonable timescale for BioSAXS measurements. The use of thin tape, carved with the VersaLaser, is a very effective fast-prototyping method for testing microfluidic designs. Regardless of the larger than expected fluctuations in concentration over time and the formation of leaks at high PEG flow rates, an enrichment factor of approximately 50% was achieved at protein flow rates of 1 μl/min into the device.

Based on these tests, a seven-layer chip was expected to provide a significantly increased protein concentration. The problem with the seven-layer device was placing tubes to allow fluid to travel to the middle tape layer without leaking. While further design refinements will be necessary to achieve an ideal 10-fold enrichment target, the existing five-layer device should serve as a good proof-of-concept test on the beamline. Also, the PDMS device had proper fluid flow, but it was difficult to correctly place the syringe in the channel, between the membrane and PDMS.

Future Work:

Future work should consist of increasing the shelf life of the device. The current device design is able to last for four to twelve hours of continuous fluid flow through the chip, and should be able to last for 36 to 40 hours of continuous fluid flow. Future device designs should have reduced leakage, by 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.

Acknowledgments:

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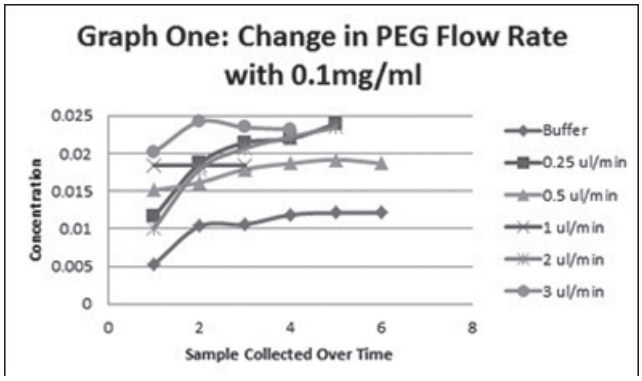
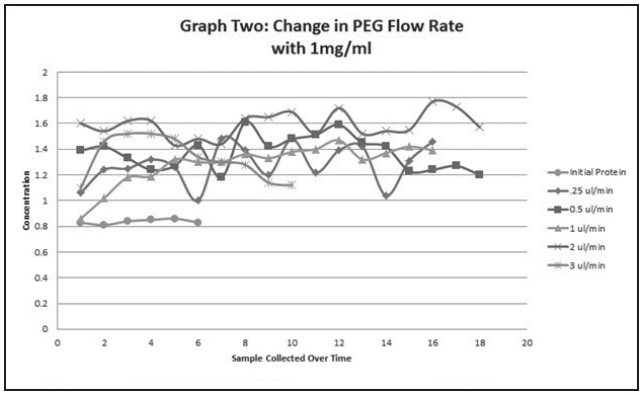
References:

[1] C. Kim, et al., "Microfluidic Dialysis Device Fabrication for Protein Solution Enrichment and Its Enrichment Enhancement by Plasma Surface Treatment of a Membrane," Journal of the Korean Physical Society, September 2007.





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.