High-Throughput Low-Volume Protein Crystallization Screening

Thesis

The goal of this research project is to create a microfluidic platform that will rapidly and automatically perform hundreds or thousands of protein crystallization experiments using only a very small volume of protein solution. The technology will be scalable for performing other chemical assays in nanovolumes at high throughout.

Introduction

Protein crystallization is one of the most important and difficult assays in molecular biology. The goal of protein crystallization in general is to grow a crystal from a protein solution, as shown in figure 1. Once a crystal is grown, x-ray diffraction may be used to determine the chemical and physical structure of the protein molecule, which reveals how the protein functions. To get good diffraction data, one needs good quality crystals. This methodology, known as "structural proteomics" is vitally important for rapidly developing new drugs.

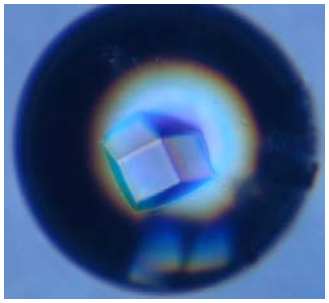

Figure 1: Image of a protein crystal grown in two nanoliters of solution. Typical crystals are grown in microliters.

Highly purified protein solution, required for crystallization, is one of the most valuable substances in existence, with some solutions worth more than one thousand times their weight in gold. Furthermore, and unfortunately, no one knows how to crystallize a protein effectively. The current method of protein crystallization involves little more than trial and error. The most common method of running protein crystallization assays is setting up a large number of experiments in a microplate. For a human, pipetting errors are likely, and the process is time consuming. Robotics can automate this process, quickly and accurately pipetting small volumes of protein solution into the microplate. However, these robotic systems, which cost hundreds of thousands of dollars and are prone to mechanical problems, can only pipette down to 20 nanoliter. Pipetting becomes inaccurate for small volumes, and impossible for smaller volumes. Also, because the high surface area to volume ratio for small volume drops, evaporation is a serious concern in microplate experiments. A solution's concentration could change considerably between the time it is deposited and the time the microplate is closed, even in under a minute. In a typical experiment using a microplate, each well in the microplate contains about one to five microliters of protein solution.

The end result of this project will be an automated protein crystallization microsystem that will operate at a fraction of the cost of a robotic system, yet at high speed and consume less protein. Furthermore, because the protein droplets will be surrounded by oil, evaporation will not be a problem. By using the dynamics of microfluidic flow of solution into oil rather than mechanics to control the volume of the protein solution droplets, high-precision sub-nanoliter droplet formation is possible. This can reduce the amount of protein solution per experiment by a factor of more than five thousand, and reducing the cost of the experiment by the same factor.

Design

The microfluidic platform consists of two main parts: a microfluidic chip and a computer controlled pressure delivery system.

Microfluidic chip

The principle of operation of the chip is illustrated in figure 2. A protein solution of desired concentration is generated by bringing protein solution and a desired chemical additive together in a microfluidic flow system. The resulting "mother liquor" is injected into a continually flowing oil stream resulting in a rapid and controllable generation of droplets. Since the entire system is microscopic, the droplets each contain sub-microliter or even sub-nanoliter volumes. Each drop is a unique chemical assay.

Since the carrier fluid is oil, the solution in the drops will not evaporate (in general), and the droplet chemistry will remain constant. Furthermore, since the entire system is hydraulic (incompressible), the droplet/oil stream can be made to stop, locking the droplets in place, by simply sealing the flow channels.

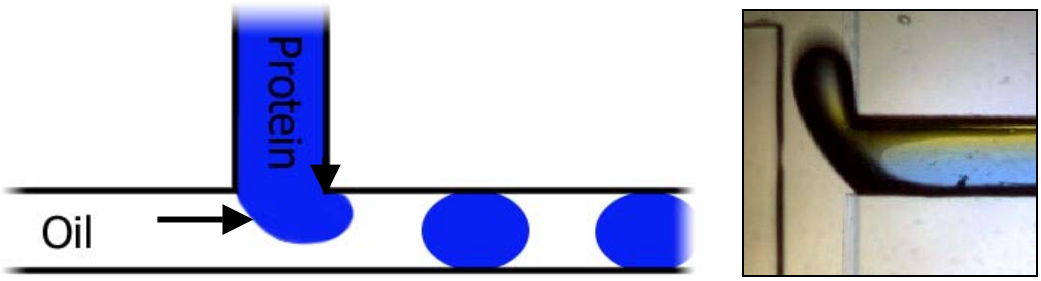

Figure 2. Protein droplet formation in oil. Droplet formation is highly controllable and very rapid. Droplets of sub nanoliter volumes can be generated at speeds of over 100 drops per second. Image on the right shows preliminary data from a microfluidic chip using this technology.

The current chip designed uses a "T" intersection to generate the solution droplets. The protein solution and oil meet at a T-intersection, both being forced forward at a steady rate. When these two immiscible fluids meet in this manner, the effect is that the protein pushes out of its channel until a large enough amount is in the line of the oil flow, and then a protein solution droplet is "pinched" off and carried away by the flow of oil. This design is capable of producing steady stream of uniformly sized droplets. To achieve sub-nanoliter droplet formation, very narrow channels are needed at this T-intersection. The smallest practical size for conventional milling is approximately 1/64 inch. To generate even smaller volumes, micromolding using silicon molds generated in the Integrated Nanosystems Research Facility clean room must be used.

The design of the chip will change considerably as this project progresses and new designs are tested, as will the fabrication and materials. The current chips are fabricated from a 2" x 2" x 0.25" piece of cast acrylic (PMMA). Using a computer numerical control (CNC) milling machine, channels are milled into the surface of the acrylic and holes are drilled to connect the pressure system.

The first chips will be made from acrylic because acrylic is transparent, inexpensive, and easy to use. However, future experiments will include the use of other materials, since acrylic is not chemically inert. Polydimethylsiloxane (PDMS) is a transparent, chemically inert polymer that will be explored to create the chip. PDMS can be micromolded to produce microfluidic sections that are under 50 microns in dimension, and can result in assay volumes in picoliters. Other materials, including epoxies and polyurethanes will also be explored.

The current design features serpentine channels to store hundreds of droplets on a 2 by 2 inch chip simultaneously (figure 3). Future designs will accommodate thousands of drops simultaneously.

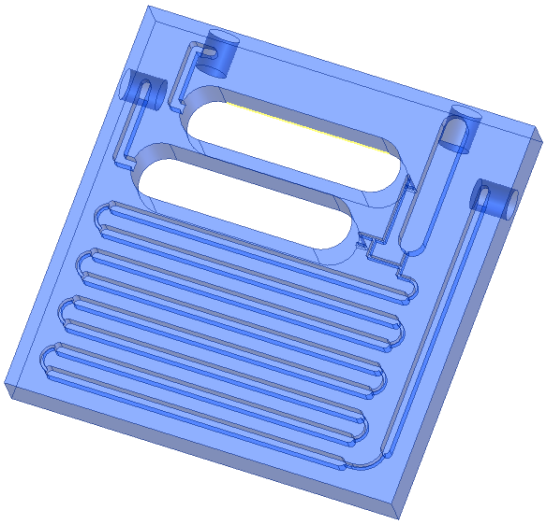

Figure 3: Design of microfluidic chip with serpentine section for storing nanodrops.

Pressure Control System

A pressure control system is necessary for controlling the rates of flow of the protein solution, additive solution, and oil. By controlling the flow rate, the chemical composition of each drop in the assay can be controlled, as can the droplet size. It is possible to control the flow rate by controlling the pressure simply by using columns of liquid to generate pressure. However, for the final product, a computer-controlled system will be developed. The pressure control system will consist of electronic pressure valves, pressure sensors, an interface for connecting to the chip, and a user interface for controlling pressure via the computer.

To provide precision computerized control, a closed-loop feedback assisted system will be employed, as illustrated in figure 4. In this system, pressure will be generated from an external air source and applied to a manifold. Electronically controlled proportional valves will allow pressurized air to escape (bleed) the manifold at a constant (controllable) rate, resulting in a controlled pressure in the manifold. A pressure sensor in the manifold connects to a circuit that compares the pressure to the desired pressure (provided by computer), and then feeds back into the proportional valve, opening or closing it as necessary. In this way, we can achieve a precise computer-controlled pressure delivery system.

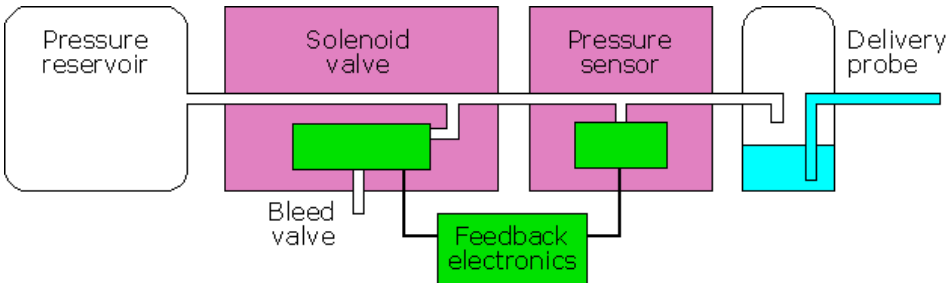

Figure 4: Diagram of operation for a computer controlled pressure delivery system.

By closing the exit channel, all flow of fluid through the chip is halted (they have no where to go). Since the fluid is incompressible, and since the flow is viscous in microchannels, droplets freeze in place allowing them to be observed under a microscope.

Methods

The efficacy of this exciting new technology for protein crystallization assays will be tested using several experiments that will be performed concurrently with the development of this platform.

The first experimental study will be an analysis of droplet size on crystal growth. Using the pressure control system to gradually increase the pressure on a lysozyme protein solution will result in a series of droplets ranging from approximately 200 picoliters to 200 nanoliters. These droplets can be assessed the see if there is a correlation between droplet size and crystallization.

Another experiment will be an analysis of the effects of different immiscible oils on crystallization process. Some oils are slightly soluble in the protein solution, and can result in some loss of the solution to the oil by diffusion. In some cases, this can help the crystallization process since it will temporarily put the protein solution in a supersaturated state. Paraffin oil is completely immiscible, whereas silicon oil is known to allow some diffusion of water. Both of these oils and their effects on protein crystallization will be studied in this platform.

By adding additional channels, it is possible to combine multiple reagents into a single droplet. Furthermore, by gradually varying the flow rates of these reagents, it is possible to create a series of droplets that vary in concentration. This exciting technology has applications throughout the fields of biology and chemistry. For this particular application, it can be used to vary the ratio of salt buffer to protein solution in a protein droplet.

Potential results

Each of the experiments outlined in the Methods section has the potential to yield important, publishable results. The platform itself has vast applications outside of protein crystallization. This method of setting up experiments is fast, accurate, and inexpensive. This platform has the potential to replace robotics in labs across the world and dramatically improve scientific throughput.

Student's Responsibility

I will design and fabricate the chips used in this project. I will assist in the development of the computer controlled pressure system, and I will write most of the computer code for the user interface. I will also carry out the experiments and data analysis necessary for this project. I will work independently on this project, with supervision from my faculty mentor and a graduate student also working on this technology. I will also prepare weekly progress reports on my activity.

Timeline

November - December	Complete designs and initial studies of droplet flow. Fabricate several small prototypes
January - February	Complete prototype pressure delivery system and computer interface. Test and demonstrate performance.
February - April	Pursue crystallization studies using protein solutions and crystallization platform.
May - June	Complete data analysis. Prepare a presentation for the UROP Spring Research Symposium.

Itemized budget

Materials (acrylic, polyurethane, PDMS, etc.)	$200
Clean room fees associated with this project	$200
Maskmaking services (for lithography)	$100

Prototyping accessories (endmills, drill bits)	$50
Lab consumables (wipes, dishes, labware)	$100
Chemicals, proteins, reagents	$200
Electronics components, PCB fabrication	$150
Total Budget:	$1000

References

Chiang Y, Bachman M, Chang H, Chu C, Li G. "Characterizing the process of cast molding microfluidic systems." *Microfluidic Devices and Systems II , Proceedings of SPIE Volume: 3877*, Chong Ahn and Bruno Frazier, Eds. August 1999.

Guidos S, Simeone M. "Binary collision of drops in simple shear flow by computer-assisted video optical microscopy." *Journal of Fluid Mechanics.* 357: 1-20, 1998.

Juarez-Martinez G, Steinmann P, Roszak AW, Isaacs NW, Cooper JM. "High-throughput screens for postgenomics: studies of protein crystallization using microsystems technology." *Analytical Chemistry*. 74: 3505-10, 2002.

McPherson A, *Preparation and Analysis of Protein Crystals*. 1982. Wiley & Sons, New York.

Rhodes G. *Crystallography Made Crystal Clear*. 2nd edition, 2000. Academic Press, San Diego, CA.

Song H, Tice J, Ismagilov R. "A Microfluidic System for Controlling Reaction Networks in Time." *Angewandte Chemie.* 42: 767-72, 2003.

Tokeshi M, Minagawa T, et al. "Continuous-Flow Chemical Processing on a Microchip by Combining Microunit Operations and a Multiphase Flow Network." *Analytical Chemistry*. 74: 1565-71, 2002.

Zheng B, Roach S, Ismagilov R. "Screening of Protein Crystaliztion Conditions on a Microfluidic Chip Using Nanoliter-Size Droplets." *Journal of the American Chemical Society.* 125: 11170-1, 2003.