Microfluidic Systems for Protein Crystal Growth

Kelly Costello
Mechanical Engineering, Corning Community College

NNIN REU Site: Minnesota Nanotechnology Cluster, University of Minnesota, Twin Cities
NNIN REU Principal Investigator: Victor Barocas, Biomedical Engineering, University of Minnesota, Twin Cities
NNIN REU Mentor: Masano Sugiyama, Chemical Engineering, University of Minnesota, Twin Cities
Contact: kellymn6@yahoo.com, baroc001@umn.edu

Introduction:

The three dimensional structure of a protein determines its interactions with other proteins as well as its properties. Being able to model and understand this structure is essential in developing drug treatments, understanding genetic mutations, and creating engineered proteins with improved or desired properties. The most common method of determining this structure is using x-ray diffraction.

X-ray diffraction involves using a large, ordered protein crystal as a diffraction grating. When x-rays are diffracted, the resulting diffraction pattern can be analyzed to yield important structural information. The current rate-limiting step in this process is the crystallization of proteins. Without crystals of sufficient size and quality, the diffraction pattern is insufficient to determine the three dimensional structure.

Crystallization of proteins relies on manipulation of the phase diagram. See Figure 1. This phase diagram relates protein concentration with any one of many other variable parameters including: temperature, solute concentration, and pH. The regions of the phase diagram include the unsaturated region where proteins exist in solution, and several regions above the saturation curve where the solution is said to be supersaturated. Within the supersaturated area, there is the metastable region, then the labile region or nucleation zone, and finally the precipitation region. In the precipitation region, an amorphous precipitate occurs, which is of no use in x-ray diffraction. The two areas of greatest concern are the labile and metastable regions. Ideally, the path taken in the crystallization process would first move into the labile region where formation of crystals occurs, and remain in the region until a desired amount of nuclei are formed. Then the solution would move to the metastable region where already formed crystals will continue to grow, but nucleation will not occur. Several methods of crystallization rely on factors such as diffusion or evaporation to create the path. The method currently used in our lab allows outside control of the movement through the phase diagram using a continuous-feed crystallization chamber. See Figure 2.

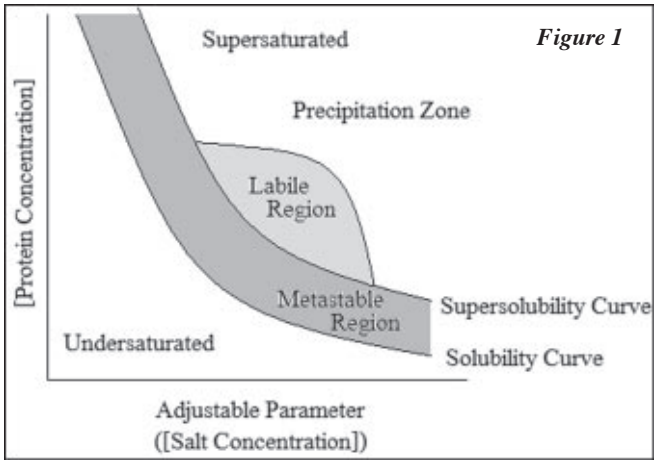

Figure 1

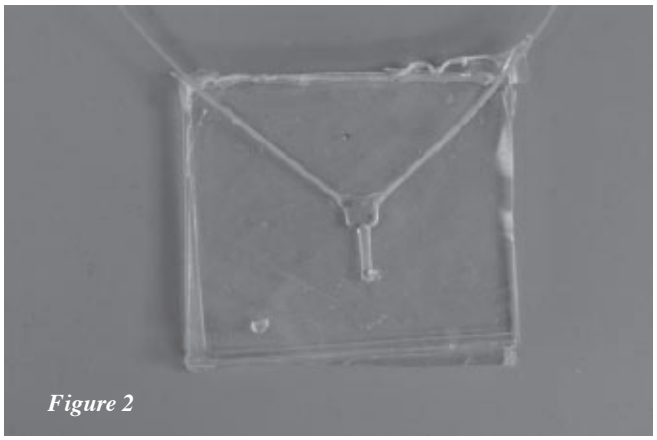

Figure 2

The rate of movement and the path taken during crystallization greatly affect the success or failure of crystal formation, but the key to crystallization of more difficult proteins lies in the nucleation stage [1]. There are two types of nucleation: homogeneous nucleation occurs within the solution, while heterogeneous nucleation occurs at the interface between the solution and its surroundings. In some cases, nucleation occurs too rapidly and produces many small crystals with too many defects for diffraction. In other cases, no nucleation occurs at all. Techniques to induce or prevent nucleation can be the key to forming large, quality crystals suitable

for structure determination using diffraction. Some of these techniques include seeding, epitaxy, or charged surfaces. The focus of our current research is inducing and controlling placement of crystal nuclei in an environment where crystals can then be easily harvested for diffraction utilizing the electrostatic charges between a positively charged protein molecule and a doped silicon surface.

Fabrication:

Continuous-feed crystallization chambers were first designed and fabricated using molded PDMS and tubing to create a new system that allows easy access to crystals formed in the chambers. Each consists of two inputs, a 2 mm x 2 mm chamber, and an output. See Figure 2. The controlled input of solution allows movement around the phase diagram relating protein concentration and solute concentration. Temperature and pH remain constant. Previous experiments were conducted in chambers made using Plexiglass® and light-cure adhesive, but the hydrophobic properties of PDMS discourage heterogeneous nucleation and allow for greater control of crystal placement as well as being less permanent.

The second stage of fabrication involves nanofabrication techniques on a silicon wafer. Starting with a p-type silicon wafer, a layer of n-type silicon is deposited on the wafer to a depth of ~ 2.5 μm. The wafer is then patterned using photolithography. The photoresist acts as a protective layer during etching, where the deposited layer of n-type silicon is selectively removed. When the resist is then removed, the result is a p-type substrate with n-type features of varying shapes, sizes, and spacings. Stripes and boxes range in size from 10-150 μm with spacings varying from 50-750 μm, while the actual growth stages are 2 mm x 2 mm for placement in crystallization chambers.

Results and Conclusions:

Crystallization experiments were conducted using a 35 mg/mL lysozyme solution and a 90 mg/mL salt solution with a 0.1M sodium acetate buffer at a pH of 4.2. Solutions were input at 0.5 μL/hr with outputs controlled to prevent evaporation in the chamber. Polydimethylsiloxane (PDMS) successfully prevented heterogeneous nucleation on the surface of the chamber, with crystals forming almost exclusively on the silicon growth stages. In previous experiments it has been determined that lysozyme molecules are attracted to n-type silicon at a pH level less than 7.0 and p-type at levels above 7.0 [2]. Our preliminary results agree with this research. See Figure 3. Crystals formed selectively on the n-type features in most cases, as seen in the figure.

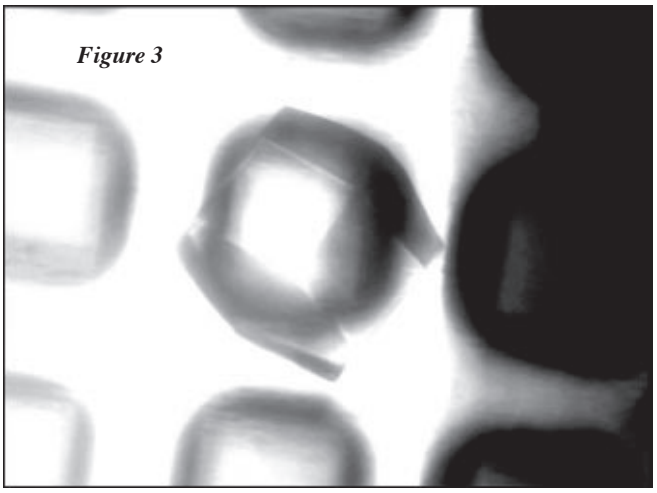

Figure 3

Future Research:

Future work is expected to include: further study of the effect of the shapes, sizes, and spacings of features; experiments conducted at varying pH levels; examination of effect of surface topography differences between deposited polysilicon versus monocrystalline silicon wafers; and tests of stages made of n-type substrate with p-type features. The hope is that the results of this research could lead to crystallization and diffraction of more difficult proteins leading to advances in pharmacology and biotechnology.

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References:

[1] N. Chayen. Current Opinion in Structural Biology. 14 (2004), p.577.

[2] A. Sanjoh and T.Tsukihara. Journal of Crystal Growth. 196 (1999), p. 691.