Toward Optimized Two-Dimensional Protein Crystallization: A Study on the Effects of Aggregation

By

James E. Young

A Thesis Submitted to the Graduate
Faculty of Rensselaer Polytechnic Institute
In Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

Major Subject: Chemical Engineering

Approved:

_____

Amir H. Hirsa
Thesis Advisor

Rensselaer Polytechnic Institute
Troy, New York
April 2012
(For Graduation May 2012)

ABSTRACT

Proteins are essential components of life as they are involved in almost all known biological functions (Glick *et al.* 2003). The specific function of a protein is heavily dependent on its three-dimensional structure at the molecular level. Having a detailed structural description of various proteins has led to many scientific advances, including the rational design of new drugs and pharmacological agents. Furthermore, our understanding of biological systems at the molecular level has been expanded due to our knowledge of the structure of proteins and nucleic acids. Developing a further understanding of protein structure could lead to the creation of functional, self-assembled nanostructures built for purposefully designed macromolecules. X-ray crystallography is the main technique used for determining protein structure. Obtaining protein crystals is therefore a fundamental aspect for protein structure studies, but it remains a challenging hurdle. The development of techniques for enhancing the protein crystallization process can significantly contribute to the advancement of the field. Also, by investigating the mechanics of protein crystallization, we gain insight in the underlying physics of many diseases including: Alzheimer's, Huntington's, Parkinson's, and sickle cell anemia, to name a few (Yan *et al*. 2004).

However, studies involving protein crystallization are often carried out with limited success. One common pitfall is the issue of protein aggregation. Aggregation, which modifies a protein's three-dimensional molecular structure, can prevent a protein from ever being crystallized. Here we present an investigation on the effect of aggregation on two-dimensional protein crystallization at the

air/water interface. We have found that upon reconstitution of commercially purchased protein, the protein solution becomes highly aggregated. This aggregation is to a degree that has proven to make protein crystallization impossible. In this study, we seek to assess the initial aggregation state of a protein that is not crystallizable, and to investigate mechanisms by which to return the protein solution to a crystallizable state by lowering the amount of aggregated protein present in the system. The ultimate goal of this study is to increase the likelihood of a successful two-dimensional crystallization experiment by increasing a given protein's susceptibility to be crystallized.