Flow Enhanced Protein Crystallization at the Air/Water Interface

By

Ali Nejatbakhsh Azadani

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Examining Committee:

Amir H. Hirsa, Thesis Advisor

Georges Belfort, Member

Angel E. García, Member

Joel L. Plawsky, Member

Timothy Wei, Member

Rensselaer Polytechnic Institute
Troy, New York

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ABSTRACT

The ability to utilize detailed knowledge of the structure of proteins and to define their interactions with ligands has enabled many advances in our understanding of biological systems and the rational design of new drugs. The primary technique that yields a detailed description of protein structure is X-ray diffraction analysis. A major challenge to the full exploitation of this powerful technique is that the protein must be first crystallized. A variety of conventional techniques are used to produce protein crystals. Most of the crystallization techniques are based on diffusion of one or more species through either liquid or vapor to bring about supersaturation. In this thesis, we introduced fluid dynamics as a novel strategy to control protein-protein interactions and induce crystallization. Two-dimensional protein crystallization on lipid monolayers at the air/water interface is a well established method. The method entails the specific binding of a protein to a fluid lipid monolayer containing a ligand. The protein studied most extensively by this method is the bacterial protein streptavidin. Streptavidin is a tetrameric protein, where each of the four subunits possess a high binding affinity to biotin.

We investigated the effects of flow in an apparatus consisting of a stationary open cylinder driven by the constant rotation of the floor. A wide range of shear is imposed by the axisymmetric flow across the air/water interface. We observed 2D protein crystallization under conditions where in the absence of flow, crystallization fails to occur. Even under conditions where crystallization does occur in quiescent systems, we have found that flow can accelerate the crystallization process. By interrogating the flow responsible for this enhanced crystallization, we have correlated the enhancement with large shear in the plane of the interface. Furthermore, we investigated the coupling between the protein-laden film and the bulk flow. In a collaborative effort, the interfacial velocities have been calculated using a standard macroscale Newtonian interface model with a variable surface shear viscosity. The results provide a macroscale description of the molecular scale processes and experimental measurements were used to test the validity of the Newtonian interface model. Despite the huge range of length scales involved, a good description of the resultant interfacial velocity field has been obtained. Moreover, in experiments with the flow started at various times following the protein injection and different ionic strength of the standard buffer solution, we observed novel protein crystals, with a very high aspect ratio, which we refer to as 1D crystals. The results demonstrate the fact that such a well-controlled flow has a great potential to enhance protein crystallization.