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Coupling between protein-laden films and a shearing bulk flow

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Abstract

Two-dimensional protein crystallization on lipid monolayers at a quiescent air/water interface is now a well-established process, but it only operates under a very restricted set of conditions and on a very slow time scale. We have recently been able to significantly extend the conditions under which the proteins will crystallize as well as speed up the process by subjecting the interface to a shearing flow. Here, we investigate the two-way coupling between a protein-laden film and the bulk flow that provides the interfacial shear. This flow in a stationary open cylinder is driven by the constant rotation of the floor. Using the Boussinesq–Scriven surface model for a Newtonian interface coupled to the Navier–Stokes equations for the bulk flow, we find that the surface shear viscosity of protein-laden films under most conditions is small or negligible. This is the case for films subjected to constant shearing flow, regardless of the duration of the flow. However, when the film is intermittently sheared, significant surface shear viscosity is evident. In these cases, the surface shear viscosity is not uniform across the film.

Keywords: Surface shear viscosity; Two-dimensional protein crystallization

1. Introduction

The study of protein transport processes in the presence of bulk flow is an emerging field. Many of the studies are focused on quantifying the transport kinetics of proteins in the bulk (liquid) or between the liquid and the gas/liquid interface [1]. These are motivated by diverse applications, ranging from replacement lung surfactant therapy [2] to food sciences, e.g. for making stable emulsions [1].

One of the underlying motivations for the current investigation is to explore the use of fluid dynamics to further our understanding of protein structure, which in turn can advance biological sciences. Further, knowledge of protein structure is the basis for the rational design of new drugs and pharmacological agents. The primary method for determining protein structure and their interaction with ligands is X-ray crystallography. However, before this powerful technique can be utilized, the protein must first be crystallized. Crystallographers acknowledge that growing crystals is often the major bottleneck in structure determination [3], and fluid dynamics has the potential to improve the situation.

The principle behind protein crystallization is that a protein solution must by some means be transformed to a supersaturated state whereby its return to equilibrium forces exclusion of protein from the solution and into a solid phase, i.e. the crystal. If the super-saturation is too small, the nucleation rate will be so slow that crystals do not form in a reasonable amount of time. On the other hand, if the super-saturation is too large, the probability of spontaneous and uncontrolled nucleation is enhanced which results in extensive and uncontrolled showers of crystals [4]. By virtue of their numbers, none of these crystals grow adequately and they are not suitable for structure studies. Therefore, active control of the conditions, for example, by reducing super-saturation once nucleation has occurred, has been a goal for crystallographers to obtain larger and better-ordered crystals.

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The current interest in 2D protein crystallization stems from the fact that in contrast to growing 3D protein crystals in the bulk, 2D protein crystallization simplifies both the theoretical and experimental aspects of protein studies. For example, 2D systems are not affected by gravity, an issue that plagues 3D crystallographers. Attempts to alleviate this include growing crystals in micro-gravity environments [5]. Another advantage of 2D crystallization is that the protein is concentrated at the interface, requiring much smaller quantities of protein. In practice, this may be crucial for the class of proteins known as membrane proteins due to the difficulty in obtaining large quantities of them. Even for water-soluble proteins, which are of current interest, the relatively high cost of the protein strongly favors 2D crystallization.

The most commonly used approach for 2D crystallization entails the specific binding of a protein to a ligand-embedded monolayer. In practice, dissolved protein is slowly injected into a quiescent aqueous pool on which a ligand-embedded lipid monolayer is initially spread. The protein crystallized most extensively is the bacterial protein streptavidin which is a well-characterized protein and has a high binding affinity to biotin (vitamin-B_7) [6–8]. Several studies have found that streptavidin can be crystallized on a biotinylated lipid monolayer [9–14]. The dissolved streptavidin in the bulk liquid binds to the ligands formed by the biotinylated lipid monolayer initially spread on the liquid surface. Due to a high concentration of protein at the interface and fluidity of the monolayer, the protein self-assembles into the crystal form.

In [15], it was observed that a shearing bulk flow can induce 2D crystallization under conditions where crystallization would not occur in the absence of flow. Here we address how such an evolving surface film, essentially a protein embedded membrane, interacts with the bulk hydrodynamics. Through detailed comparisons between velocity measurements of the protein (crystals and clusters) at the interface and numerical solution of the Navier–Stokes equations coupled to a Newtonian interface (Boussinesq–Scriven surface model), we show that the surface model is applicable over a wide range of conditions.

The flow system utilized is one which we have used previously to study the hydrodynamic coupling between Langmuir monolayers and swirling shear flows with inertia [17,18]. To maximize the signal-to-noise ratio, the experiments were performed at nearly the highest Reynolds number for which the macroscale bulk flow remains axisymmetric. Although high Re flow is not typical of biological systems with protein embedded films, the observed macroscopic response of the protein embedded film to shear (e.g., as expressed by the surface shear viscosity μ^s) is expected to be the same as long as the film remains Newtonian. The flow geometry, namely a stationary cylinder with a free surface, driven by the constant rotation of the floor, is shown in Fig. 1. This flow geometry was selected, in part, because it allows easy optical access to a relatively large portion of the air/water interface. Also, the circular interface with large area-to-perimeter ratio is particularly advantageous in experiments as it minimizes any meniscus effects.

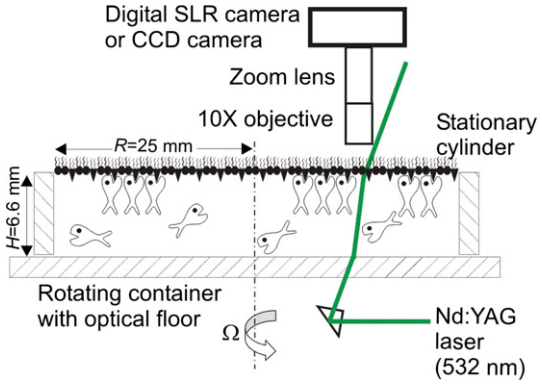

Fig. 1. Schematic of the experimental set-up. The protein crystals (and/or clusters) are illuminated by forward scattering of a laser. The schematic representations of the protein as fish, the ligand as triangles, and diluting lipid as circles, were borrowed from [16].

2. Experimental procedure

The experiments were conducted using the same flow apparatus described in [19]. Aside from its basic features, here we will describe the modifications made to the apparatus for experiments with protein. The flow apparatus consists of a stationary cylinder, made of precision bore glass, with a diameter of $2R = 5.0$ cm and height $H = 0.66$ cm. The cylinder was held by a piece of precision machined Delrin. The Delrin aligned the cylinder axis to within 0.004 cm of the floor axis of rotation. The gap between the bottom of the stationary cylinder and the rotating floor was kept less than 0.008 cm. The rotating floor consisted of an optical quality glass window and made true to within 0.002 cm. Not shown in Fig. 1 is a glass cylinder with larger height and diameter than the stationary cylinder which was bonded to the rotating optical floor in order to form a water-tight rotating container. Thus, there was no need for a seal between the stationary cylinder and the rotating floor.

The use of the Delrin holder for the stationary cylinder along with all the remaining pieces which were made of glass, allowed for thorough cleaning of the surfaces that came in contact with the fluid system between each experiment. Prior to each experiment, the flow apparatus was washed using RBS detergent (PIERCE, catalog no. 27959) to remove any residual protein, then rinsed at least fifteen times with Milli-Q filtered de-ionized water (Millipore Inc.). After the system was allowed to fully dry, high-purity solvent cleaning was performed with acetone. The solvent cleaning procedure was then repeated with methanol, and Milli-Q filtered water. The cleaning prior to each experiment and the use of high-purity materials for the experiment, including water, salt, monolayer forming materials, etc., ensured minimal contamination of the experiment.

For the preparation of the buffer and the monolayer mixture, the procedures in [12,14,20] were followed with some modifications. The experiments were conducted using an aqueous buffer prepared with de-ionized water (Milli-Q filtered, with resistivity greater than 18 MΩ cm). Salt (NaCl, >99.999%, Sigma-Aldrich, catalog no. 204439) was added to make a solution with concentration of 500 milli-molar (mM). Subsequently, sodium phosphate monobasic (NaH_2PO_4, Reagent Plus grade,