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December 9, 2003

Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Stevenick
Biocomplexity Institute
Indiana University
Swain Hall West 117
Bloomington, IN 47405-7105

Dear van Stevenick,

I am responding to the advertisement for a tenure-track position at the assistant professor level in support of the Biocomplexity Institute at Indiana University. Please find enclosed my application (curriculum vitae with a list of publications and a statement of research interests). My letters of reference are from the following three scientists:

1. Prof. George B. Benedek
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2. Dr. William A. Eaton
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I look forward to hearing from you.

Sincerely,

Neer Asherie
Research Associate

Neer Asherie

Research Interests

Proteins in solution crystallize, unmix, aggregate and gel (Fig. 1). Since these phenomena all involve general, collective protein interactions, they are often referred to as "phase behavior". I want to study the phase behavior of protein solutions and understand how it arises from the interactions between proteins.

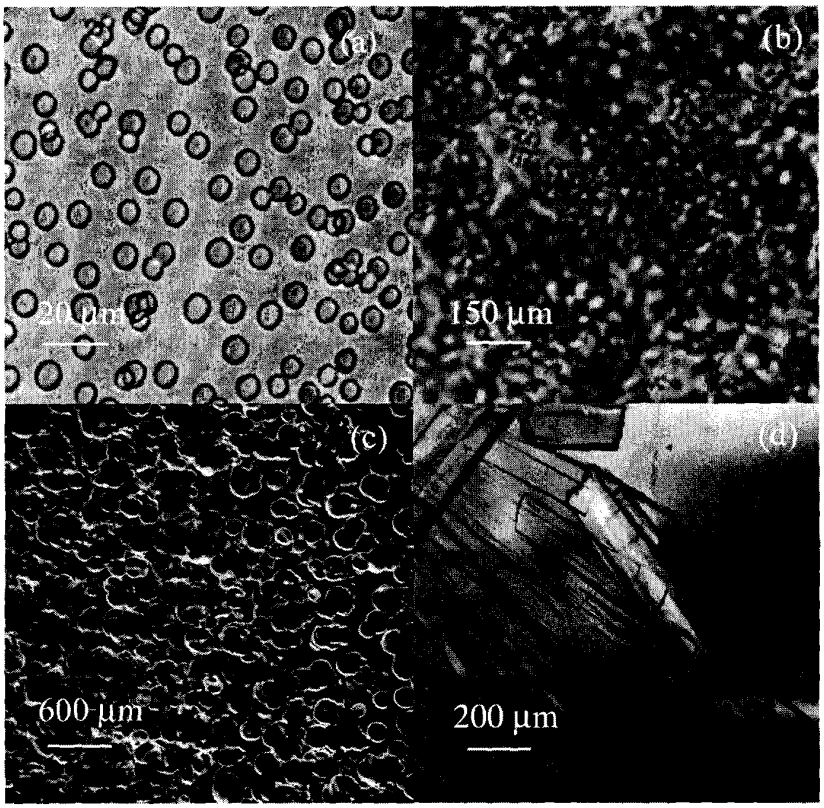

Figure 1. Transformations in protein solutions. These transformations occur when the conditions, such as temperature and pH, are changed for an initially homogeneous protein solution.
(a) Liquid-liquid phase separation ("unmixing"): the drops have a high protein concentration, while the background is at low protein concentration;
(b) Aggregation: disordered flakes of protein in solution;
(c) Gelation: clusters of protein linked together;
(d) Crystallization: protein crystals in solution.

Why is it important to study how proteins crystallize, unmix, aggregate and gel? One reason is that protein phase behavior governs many diseases. For example, cataract involves the liquid-liquid phase separation, aggregation and crystallization of lens proteins [1]. Sickle-cell anemia is caused by the gelation of a mutant hemoglobin [2], while Alzheimer's is caused by the aggregation and fibrillization of beta-amyloid protein [3].

There are other phenomena besides diseases for which protein phase behavior is important. For instance: (i) the effective release of certain drugs, such as insulin, depends on the particular state of the protein [4]; (ii) in industry, liquid-liquid phase separation and crystallization are used to purify proteins [5]; (iii) the compartmentation of the cell cytoplasm is thought to be driven, at least in part, by phase separation [6]; and (iv) protein crystallization produces the crystals from which the x-ray structure of proteins are determined [7].

If we wish to tackle these processes so as to improve them, for instance, to fight certain diseases or produce high quality crystals for x-ray studies, a fundamental understanding of protein phase behavior is needed. This understanding does not currently exist, but my proposed research will help to fill in this gap in our knowledge about proteins.

My research will improve the understanding of the phase behavior of protein solutions by addressing the following questions:

1. *Does a general phase diagram exist for aqueous protein solutions?*

2. *What is the connection between model protein interactions that describe the phase behavior and real protein interactions?*

3. *How does the phase behavior of membrane proteins differ from that of water-soluble ones?*

A detailed description of the research with a discussion of these questions is given below.

1. PHASE DIAGRAMS OF AQUEOUS PROTEIN SOLUTIONS

The phase diagram is a map which gives the state of the material (e.g. solid vs. liquid) as a function of the ambient conditions (e.g. temperature). It has therefore proven to be a useful tool in processing many different classes of materials, such as simple fluids, metal alloys, colloidal dispersions and synthetic polymers. For each of these classes of materials there are just a few types of phase diagram. This implies that simple models should be able to describe the interactions which lead to the observed phase behavior. For example, a two-parameter model (hard spheres with a given range and strength of interaction) is sufficient to adequately describe the phase behavior of most non-polar simple molecules [8]. If it can be shown that protein phase diagrams, like those of other systems, can be classified into a few types, then the idea that the phase behavior of proteins can be described by simple models for the protein interactions will be greatly strengthened (see Section 2). Furthermore, the way protein solutions are handled and studied will be simplified, just as a knowledge of phase behavior has improved the processing of other classes of materials.

It is still not known what types of phase diagrams can exist for aqueous proteins solutions since very few phase diagrams have been determined. Indeed, there are only two protein systems for which relatively complete phase diagrams have been established: lysozyme and the γ crystallins. My work and that of others has shown that both lysozyme and the γ crystallins exhibit two principal phase transitions: crystallization and liquid-liquid phase separation. This latter transition is the separation of a protein solution into two phases of unequal protein concentration which occurs upon an appropriate change of solution conditions (such as temperature, ion concentration or pH). It has been suggested that since the phase diagrams of these two systems are very similar, their phase diagram is the generic one for aqueous protein solutions [9]. This suggestion assumes that liquid-liquid phase separation, which is related to the average attraction between proteins, should be a general feature of many protein solutions. However, this transition has only been thoroughly documented for lysozyme and the γ crystallins. Since liquid-liquid phase separation is metastable with respect to crystallization, and therefore not easily seen, only a systematic investigation of protein phase diagrams can reveal whether this transition is truly as general as is believed.

I plan to determine the phase diagrams of a significant number of aqueous proteins. The candidate proteins I have identified include β-lactoglobulin, thaumatin, concanavalin A and catalase. All of these proteins are commercially available and have been crystallized from the commercial sources. It has been claimed that these proteins also undergo liquid-liquid phase separation, but no detailed measurements of their phase diagrams have been made. This experimental work will establish whether there is indeed a general phase diagram for aqueous protein solutions.

2. MODEL AND REAL PROTEIN INTERACTIONS

The phase behavior of proteins is unusually rich. As stated previously, proteins can crystallize, unmix, aggregate and gel [10]. Therefore, a large amount of information about the interactions between proteins can be extracted by examining the phase behavior of protein solutions. Until recently, there were no models for the interactions between proteins which were capable of accurately describing the thermodynamic behavior of protein solutions. However, I have helped to show that by modeling the

protein interactions as short-range and anisotropic, a correct description of the phase boundaries of lysozyme and the γ crystallins is possible [11,12]. I plan to use and expand upon these microscopic models to extract the values of the parameters (such as the range and the degree of anisotropy of the protein interactions) which reproduce the phase diagrams of other proteins.

I propose to establish this relation by studying the shifts in the phase boundaries brought about by different solution conditions and by mutations of specific protein residues. Both factors lead to shifts since they modify the protein interactions. The shifts in the phase boundaries also correspond to changes in the values of the parameters of the models. Since it is fairly well known how the real interactions of proteins change with conditions and mutations [13], a relation between the real and model interactions can be established. It will then be possible to predict the phase diagram of a protein from its real interactions. This knowledge can then be used to identify the conditions which will favor or prevent any particular phase change.

3. INTEGRAL MEMBRANE PROTEINS

Integral membrane proteins are a group of biologically important proteins which are part of a lipid membrane. Little is known about how the phase behavior of these proteins affects membrane function. Also, integral membrane proteins are difficult to crystallize and hence few three-dimensional structures of this type of protein have been determined [14]. An understanding of the phase behavior of membrane proteins would be a big step towards improving their crystallization.

Since membrane proteins are insoluble in water, they must be solubilized with an appropriate detergent in order to be studied. This introduces complications absent in aqueous systems, for the phase behavior of the detergent must then be taken into account when manipulating membrane proteins. However, despite the presence of a detergent, it has been found that membrane proteins can be crystallized by the same techniques used for water-soluble proteins [15]. Furthermore, membrane proteins can form various fluid and ordered phases, exhibiting a rich phase behavior similar to that of aqueous protein solutions [16]. These findings are not surprising for the same type of short-range and anisotropic protein interactions govern crystallization in both systems.

I plan to examine the phase behavior of several membrane proteins in an analogous fashion to the investigation of the aqueous systems proposed previously. I will begin by studying porin and maltoporin, two channel-forming proteins in the outer membrane of bacteria. These proteins are relatively easy to crystallize and some information about their phase diagrams is already available. This work will lead to a better understanding of the phase behavior of membrane proteins and so facilitate their crystallization.

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