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The Physics of Protein Crystallization

PETER G. VEKILOV[1] AND ALEXANDER A. CHERNOV[2]

[1]*Department of Chemical Engineering, University of Houston, Houston, TX 77546, USA*
[2]*Universities Space Research Association, Marshall Space Flight Center, SD46 Huntsville, AL 35812, USA*

I. Introduction

Proteins are the elementary building units of all living creatures and essential components for information and energy processing within the living systems.[1] The correlation between the structure and the function for this group of natural compounds has been the focus of intense investigations for more than 50 years. (For a brief history, see Ref. 2, and for further and more recent developments see Refs. 3–6.) Currently, the two most broadly used methods of protein structure determinations are x-ray crystallography and Nuclear Magnetic Resonance (NMR). NMR is limited to proteins of molecular mass lower than 30,000–60,000 Da, involves rather long data collection times,[7–9] and thus is expected to

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contribute at most 20% of the data. On the other hand, due to recent advances in protein expression and isolation,[10] data collection on specialized high-intensity synchrotron beam lines,[11,12] crystallization screening techniques,[13–18] and computational methods for structure determination and refinement, x-ray crystallography is close to becoming a high-throughput method.[10]

To resolve atoms that are, typically, 1.5–2 Å apart, the diffraction methods require single crystals that are as large as several tenths of a millimeter in all three dimensions, and have low defect contents and high compositional and structural uniformity. At present, most proteins can be enticed to produce some kind of microcrystals. However, the crystals are often of significantly lower quality and/or size than needed for the desired resolution. Often, further improvement in crystallization involves the application of elaborate, protein-specific techniques. A few non-exhaustive examples include genetic engineering of intermolecular contacts,[19,20] proteolysis,[21] utilization of the phase behavior of complex, multicomponent systems,[22,23] or even costly microgravity experiments aboard spacecraft.[24–28] Hence, claims that protein crystallization is a major rate-limiting step in structure determinations

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abound, even in recent literature,[5,29–32] and reflect the fact that a determined effort that will eventually produce suitable crystals may take several years.

Beyond protein single crystal growth, progress in various biochemical and biomedical research and production tasks is impeded by lack of insight into protein nucleation and growth mechanisms. For instance, the slow dissolution rate of protein crystals is used to achieve sustained release of medications, such as insulin, interferon-α, or the human growth hormone.[33–37] Work on the crystallization of other therapeutically active proteins—e.g., antibodies for foreign proteins—so they can be dispensed in a similar manner, is currently underway. If the administered dose consists of a few, larger, equidimensional crystallites, steady medication release rates can be maintained for longer periods than for doses composed of many smaller crystallites. To achieve such size distributions, crystal nucleation times must be short so that all crystals grow at the same decreasing supersaturation.

Other, more rare biomedical applications relying on insight into the formation of protein solid phases include situations where pathological conditions are related to the formation of crystals or other ordered solid aggregates in the human body. Two often-cited examples are the crystallization of hemoglobin C and the polymerization of hemoglobin S, which cause, respectively, CC and sickle cell diseases.[38–40] Another example is formation of a denser liquid phase in the eye retina, which underlies the pathology of cataract formation.[41]

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Of all processes of molecular self-assembly in the biological world, the crystallization of the protein molecules is probably the best-studied example. Other examples include the formation of quaternary protein structures, protein and protein nucleic acid complexes whose assembly into structural units is often essential to chains of coupled functions, and assembly of viri and of complex intracellular organelles such as the ribosome.[42–44] Similar to crystallization, the self-assembly processes are enabled by molecular recognition. Hence, insight into the molecular mechanisms of protein crystallization could provide guidance into these related areas.

In addition to its medical and biotechnological significance, protein crystallization has much in common and may provide an insight into crystallization phenomena that occur in a variety of systems,[45] such as water freezing in clouds and oceans,[46] magma solidification in the Earth's interior,[47] the pulling of semiconductor boules,[48] and so on. Given the resolution limits of modern surface characterization techniques, proteins are particularly attractive for studies of fundamental crystal growth mechanisms. For example, the size of the protein molecules (a few nanometers) and the time-scales for growth (up to a few seconds between sequential discrete molecular attachment events) are within the reach of current advanced experimental techniques. On the other hand, the molecular masses typical of most protein molecules still leave the thermal equilibration times relatively short. Thus, conclusions drawn from studies of protein model systems may still be meaningful for small molecule crystallization. In this regard, proteins could be a better model than, for instance, colloidal crystals.[49,50]

The preceding factors led to the emergence of macromolecular crystallization as a distinct area of research in the early 1980s. Since then, the field has benefited from concepts and methods developed in other research areas. For instance, the application of direct light scattering and other methods used to probe colloids led to quantitative measurements of molecular interactions and crystal nucleation in protein solutions.[51–55] Fluid dynamics analyses were applied to characterize the convective–diffusive supply fields in the solutions from which the crystals

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