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Guest Commentary

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Guilt by Association: The Physical Chemistry and Biology of Protein Aggregation

The aggregation of proteins and polypeptides is a major problem in the laboratory and in biotechnology, and is a serious issue in biology and human health.[1−4] Protein aggregation is a topic that is ripe for investigation by physical chemical and biophysical methods, as well as by molecular dynamics (MD) simulations, and by more coarse grained modeling and analytical theory. Much recent attention in the physical chemistry community has focused on the process of amyloid fibril formation, motivated in part by its role in human disease. More than 30 different diseases involve amyloid formation, including devastating neurodegenerative disorders, but the mechanism of amyloid formation is not well understood, and there are very few viable therapeutic approaches. Amyloids are partially ordered aggregates of proteins that contain significant β-sheet structure arrayed in a cross-β structure in which the individual peptide chains are oriented perpendicular to the long axis of the fibril (Figure 1). The formation of amyloid is a fascinating and challenging problem in molecular self-assembly. However, it is worthwhile reminding ourselves that the importance of amyloids is directly related to their role in human disease, and thus experiments and simulations conducted under simplified conditions need to connect to biology to remain relevant. Fortunately, recent developments in methodology and experimental design, as well as growing collaborations between biophysicists and biologists hold the promise of providing a more rigorous, biologically relevant description of amyloid formation. Several of these advances are highlighted in the Perspectives included in this issue. Protein aggregation is not limited to amyloid formation, and new methods and approaches are also needed to study nonamyloidogenic aggregation. For example, considerable resources are invested in optimizing protein solubility and preventing aggregation of potential therapeutic proteins. Aggregation and poor solubility have limited the development of many monoclonal antibodies and other protein-based drugs and can lead to problems with immunogenicity, as well as loss of active protein.[4] Protein aggregation can take other guises in biology beyond just amyloid. A classical example, and still arguably the best understood case of pathological protein aggregation, is the polymerization of sickle cell hemoglobin to form polymeric fibers. Elegant spectroscopic measurements together with detailed modeling have defined the mechanism of sickle cell polymerization, illustrating the power of physical chemical approaches.[5] There are numerous other examples of deleterious aggregation that does not involve amyloid, including interesting mechanisms by which certain viruses subvert host defenses.

Protein aggregation and/or polymerization in vivo and in vitro is not always bad. The discovery of functional amyloids highlights the beneficial effects of controlled amyloid formation in vivo.[3] Recent work also spotlights the importance of controlled aggregation and self-assembly in cell signaling, the generation of signalosomes, the production of other intracellular bodies, and the potential role of liquid−liquid demixing transitions in biology.[6,7] The controlled self-assembly of designed peptides into biocompatible hydrogels provides one example of the benefits of controlled aggregation in vitro, as does the design of amyloid-inspired biomaterials.[8] At an even more basic level, we still lack a general, predictive theory of protein solubility. In contrast, the interactions that control protein stability are generally well understood, and there are well-documented approaches for rationally enhancing protein stability, but understanding protein solubility is arguably even more important.

One of the most significant challenges in the study of amyloid formation is that the kinetics of self-assembly are complex and likely involve distributions of heterogeneous oligomers and possibly multiple pathways. In addition, there are

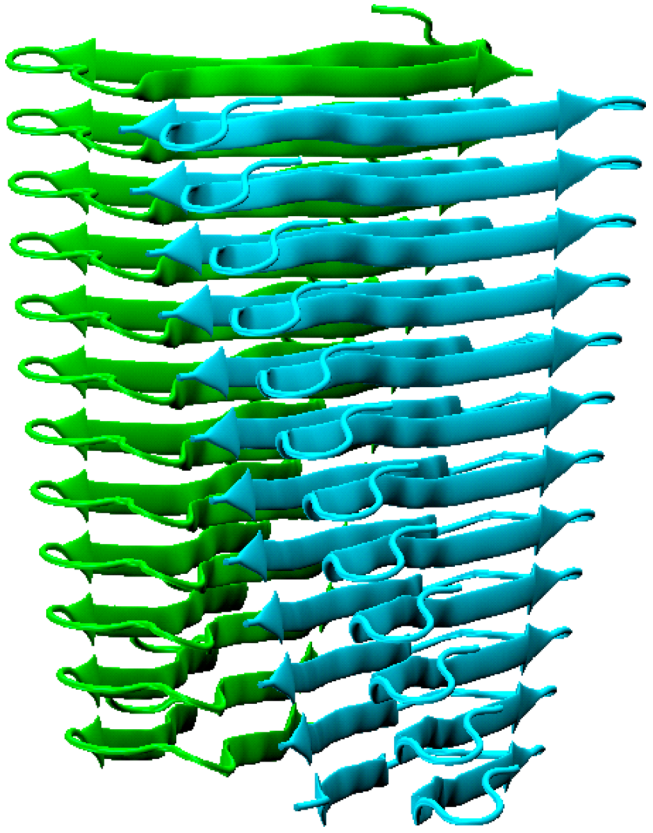

Figure 1. A ribbon diagram of a model of the basic unit of an amyloid fibril. The structure is built up of two symmetry-related stacks of U-shaped monomers. One stack of monomers is colored light blue in the cartoon, and the other is green. The β-strands run perpendicular to the long axis of the fibril. In this model, the hydrogen bonds are between adjacent peptide chains and not within a single chain. This basic structure is believed to be the core component of the amyloid fibrils formed by the Aβ peptide and the IAPP peptide.

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few high-resolution structures of amyloid fibrils, although exciting progress is being made in this area.[2,9] Furthermore, amyloids are often polymorphic. These difficulties have motivated the development of new experimental and computational approaches to study amyloid formation. The Perspectives included in this issue highlight some of the advances that are taking place in the study of protein aggregation. Amyloid formation in vitro is normally described by a sigmoid progress curve, composed of an initial lag phase in which little or no amyloid is formed, followed by a growth phase that leads to the generation of amyloid fibrils, and finally a plateau or saturation phase (Figure 2). The lag phase can be bypassed by adding "seeds" of preformed fibrils. Amyloid formation involves both primary nucleation and secondary nucleation.

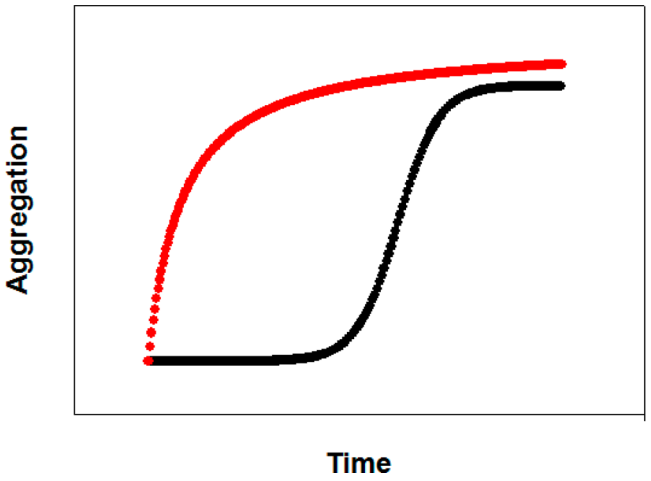

Figure 2. A schematic presentation of the time course of amyloid formation (black). A lag phase is observed, followed by a grown phase that eventually reaches a plateau. The addition of small amounts of preformed amyloid fibrils, "seeding", leads to a bypassing of the lag phase, depicted in red.

Much of our knowledge of amyloid formation comes from kinetic experiments that indirectly monitor the extent of amyloid formation using extrinsic dyes, such as thioflavin-T, that bind to amyloid fibrils. These studies, while enormously useful, do not reveal the important details of the processes that occur in the lag phase, and they provide no information about the conformation of preamyloid oligomeric species. This is particularly unfortunate as preamyloid species are thought to be the most toxic entities in a number of amyloid diseases.[10] In addition, these sorts of assays can give false positives in studies of inhibitors. For example, putative inhibitor may reduce the dye signal by competing for binding to the amyloid fibrils or by interfering with the spectroscopic readout of the dye assay instead of by reducing the amount of amyloid. Thus, there is clearly a need to develop methods that allow a keener view of the important early events in amyloid assembly. The Perspectives in this issue are focused on exactly this issue; they describe new developments that allow amyloid formation to be probed in more depth and in more detail. Shea and colleagues summarize advances in all-atom MD simulations and coarse-grained approaches, while Moran and Zanni describe advances in infrared (IR) spectroscopy and isotopic labeling, that hold promise for developing a detailed picture of the mechanism of self-assembly. Ramamoorthy and co-workers describe developments in NMR that facilitate the study of the transient species formed during amyloid formation. A key aspect of both the NMR and IR studies is that they offer nonperturbing methods for following amyloid formation in real time and can provide information about the specific structures formed. This is particularly important because, as noted, much of the data in the field comes from measuring kinetic curves using extrinsic dyes as probes.

The proteins and polypeptides that form amyloid can be broadly divided into two structural classes; on the one hand are globular proteins that adopt a compact structure in their unaggregated state, and on the other are those that are flexible and intrinsically disordered in their unaggregated state. The former typically need to undergo an unfolding or partial unfolding transition prior to aggregation. Prominent examples of globular proteins that from amyloid in vivo include β2-microglobulin and transtherytin (TRR). The molecular basis for their aggregation has been extensively studied, and detailed molecular-level pictures are emerging. The case of TRR is particularly important because the work has directly led to the first clinically approved, rationally designed drug that targets amyloid formation.[11] This extremely exciting development resulted from years of careful physical chemical, biophysical, and biochemical studies of the amyloid formation pathway of TTR, all of which were supported by careful biology studies. The work emphasizes the importance of basic physical chemical and biophysical investigations in the amyloid field and highlights the importance of making strong connections to the underlying biology. Important examples of amyloid formation by intrinsically disordered proteins include the various isoforms of the Aβ peptide in Alzheimer's disease, the tau protein in Alzheimer's disease, α-synuclein in Parkinson's disease, and islet amyloid polypeptide (IAPP, amylin), the protein component of the islet amyloid found in type-2 diabetes.

The Perspectives by Shea and co-workers and by Ramamoorthy and colleagues are focused on IAPP, while the article by Moren and Zanni describes IR studies of IAPP and other proteins. IAPP is a fascinating molecule and a very challenging experimental system. Amyloid deposits in the pancreas were first reported in 1901, but it was not until more than 80 years later, in 1987, that the protein component of islet amyloid was described.[12−14] The culprit, IAPP, is a 37 residue polypeptide hormone. In normal individuals, soluble IAPP plays a role in regulating metabolism, but it forms pancreatic amyloid by an unknown mechanism in type-2 diabetes.[15,16] Pancreatic amyloidosis leads to β-cell dysfunction, β-cell apoptosis and cell death, as well as islet transplant failure.[15,16] The peptide is extremely amyloidogenic, and it aggregates aggressively in vitro; indeed, it is even more prone to aggregate than Aβ. These factors make it challenging to study, and thus, IAPP self-assembly is less well-defined than that of Aβ. The developments and new methods highlighted in the three Perspectives in this volume, together with other advances, hold the promise of shedding more light on amyloid formation by IAPP and other proteins. Many of the methods and approaches outlined in these articles can be applied to more general problems in protein aggregation.

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