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Folding of Genetically Engineered Beta-Sheet Forming Fibrillogenic Polypeptides

The long-term goal of the proposed research is to identify the crucial intermolecular interactions involved in the folding and fibrillation of proteins. β -Sheet folding in large synthetic, fibrillogenic polypeptides will be investigated by establishing the primary sequence-property relationship governing β -sheet nucleation and the role of aggregation on formation of β -sheet nucleus. The mechanism of folding will be determined using deep UV resonance Raman (DUVRR) spectroscopy combined with advanced statistical analysis including 2D-correlation spectroscopy, independent component analysis (ICA) and pure variable methods. The variation of polypeptide length and selective site-specific introduction of β -hairpins will enable determination of the role of intra- and intermolecular folding initiation. The selective control of amino acid sequence possible by using genetically engineered polypeptides as models for native protein folding will enable careful investigation of the influence of polypeptide charge and electrolyte interactions on folding, both properties associated with the in vivo folding of amyloidogenic proteins. With a firm understanding of the influence of primary sequence on folding, the effect of templating will be investigated. As a rule, the effect is limited to templates prepared from the same protein or from a fragment with the same fibrillogenic amino acid segment. Our goal is to understand what changes in an aggregate structure compromises its ability as a template.

In preliminary work, we have established that a genetically engineered polypeptide replicates the folding and aggregation performance of more complex native proteins. The pH and concentration dependence of folding was nearly identical to that found with natural substrates. Pure variable and non-negative ICA methods have been successfully applied to the analysis of protein secondary structure based on DUVRR spectra. The combination of 2D correlation analysis with deep UV Raman spectroscopy was used to probe protein structural rearrangements at early stages of fibrillation.

The preparation of novel de novo engineered polypeptides, affords a multivariate approach to examination and investigation of the fundamental problems of protein folding and may have implications to the role of tertiary contacts and nonspecific, Flory-type coil-to-global protein collapse in folding. This work addresses the fundamental biophysics behind folding processes and may lead to insights, made possible by the use of simplified model compounds that may also have relevance to the basis for disease states with protein misfolding etiology.

The broader impact. The proposed program trains students, both graduates and undergraduates, in the most advanced modern methods and techniques, including the deep UV Raman, circular dichroism, fluorescence and absorption spectroscopies, atomic force microscopy, dynamic light scattering as well as genetic engineering and protein expression and purification. This cross-disciplinary project engages students in biophysics, biochemistry and molecular biology, preparing future professional scientists and engineers with multidisciplinary backgrounds and versatile skills. The PI and Co-I have involved the following undergraduate students from historically underrepresented groups (African-American, Latino, and Native

American) Taurean Howard, Qaid N Foulks, Prince Acheampong, Justice Agyei , Fedena Fanord, and Keesha Smith in studies related to the proposed work. The PI will integrate the advanced knowledge acquired through this NSF funded program into undergraduate and graduate courses, including CHM225-Analytical Chemistry, CHM430 – Instrumental Analysis, CHM450-Forensic Chemistry, CHM550A-Advanced Forensic Chemistry, and CHM544-Theory and Techniques of Biophysics and Biophysical Chemistry, an advanced course, for graduate/undergraduate (senior) students from Chemistry, Biological Sciences, Physics and College of Nanoscale Science and Engineering. The product of the proposed research clearly has direct applicability to understanding the etiology of protein deposition diseases, such as the neurodegenerative diseases Alzheimer's, Parkinson's, and Creutzfeld-Jacob diseases. A clearer understanding of fibrillation processes can also be utilized to develop therapy such as improving hemodialysis in treatment of dialysis related amyloidosis(DRA), a complication of long term hemodialysis required for end stage renal disease patients.