Exploring In Vitro and In Vivo Protein Aggregation: The Case of a β-Clam Protein

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For several decades now, scientists have learned a whole lot about how proteins fold. Many of these studies involve watching how a protein from a sequence of amino acids is able to find its three dimensional fold- in a test tube. But today, the real challenge is to study how proteins behave in a cellular environment consisting of very complex interactions. More interestingly, we also have very little knowledge of what happens when a protein fails to fold. What happens if protein folding is messed up?

Protein aggregation is a consequence of a protein's inability to fold. During aggregation, proteins form higher order structures that are insoluble in solution. More importantly, aggregation has been implicated in several diseases (e.g. neurodegenerative diseases, diabetes) and also poses huge challenges in large-scale production of protein therapeutics. We are interested in answering the fundamental questions: Why do proteins aggregate? Are there sequence determinants that drive a protein to aggregate and how are they related to the folding of a protein? What are the species that lead to aggregation?

We are exploring protein aggregation using a model protein, Cellular Retinoic Acid Binding Protein I (CRABP I) in the context of E. coli as a cellular model. CRABP I, a predominantly β-sheet protein is highly soluble when overexpressed in E. coli. Interestingly, introducing point mutations drives the protein to aggregate. Using hydrogen exchange, NMR and other biophysical methods we have investigated the mechanism of aggregation in these CRABP I mutants. We have found that the population of an aggregation-prone near-native intermediate lead to both in vitro and in vivo aggregation of CRABP I. Furthermore, regions of the protein that are found to form the aggregation cores are also sequences that are buried in the initial steps of folding suggesting possible common sequence determinants shared in both protein folding and aggregation.