

## “Kinetics, thermodynamics, and insights into nonnative protein aggregation”

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### **Abstract**

Non-native aggregation is a ubiquitous problem during biopharmaceutical product formulation and process development. The presence of even relatively small quantities of soluble or insoluble, nonnative aggregates may significantly increase product development time and expenses, raise regulatory concerns, and potentially jeopardize patient safety due to increased immunogenic response. The process of nonnative aggregation has also garnered significant interest from the medical and life science communities due to its potential role in generating toxic intermediate- to high-molecular-weight oligomers implicated in a growing number of chronic diseases such as Huntington’s Disease, Alzheimer’s Disease, and prion diseases.

At a minimalist level, nonnative aggregation involves multiple, parallel and sequential stages: (partial) unfolding or refolding of initially native or unfolded protein; nucleation or formation of the smallest aggregates that are (net) irreversible and stabilized by  $\beta$ -sheet secondary structure motifs; aggregate growth via polymerization (monomer addition) and condensation (aggregate-aggregate assembly) to form filaments, fibers, gels, and/or precipitates. Although much has been learned at a qualitative level about the mechanism of non-native aggregation over the past three decades, a number of fundamental, mechanistic questions remain unanswered. This is due, at least in part, to experimental difficulties with isolating or identifying key intermediates in one or more of the above stages, and to difficulties in interpreting biophysical characterization data and the effects of experimental variables such as temperature, pH, protein concentration, and excipient levels. Concomitantly, direct and predictable experimental control of the overall kinetics of nonnative aggregation remains an outstanding problem both in biopharmaceutical product development and in design of pharmaceutical molecules that inhibit the onset or progression of aggregation-mediated diseases.

This seminar will present some of our recent experimental and theoretical efforts to gain an improved qualitative and quantitative understanding of the overall mechanism of nonnative aggregation, as well as development of improved quantitative, mathematical models for predicting aggregation rates and mechanistically interpreting experimental data. The resulting kinetic model incorporates inputs from both experiment and coarse-grained statistical mechanical and molecular models, and seeks to qualitatively and quantitatively bridge gaps between traditional measures of protein stability – e.g., protein-protein interactions, folding- unfolding dynamics, and folding thermodynamics – and the macroscopic kinetics and thermodynamics of aggregate formation.