

CMET Seminar:

- › November 4, 2008
- › 1:30 P.M.
- › 366 Colburn Laboratory



Georges Belfort

Russell Sage Professor of Chemical & Biological Engineering
Rensselaer Polytechnic Institute

“Insight into the Kinetics of Oligomer Formation During Amyloidosis”

Although the critical causative proteins for more than 20 amyloid diseases have been identified, the process and mechanism by which these proteins induce disease are unknown. What is known is that these proteins are converted to cross- β -sheet rich fibril structures called amyloid fibrils. For over 100 years, the presence of these fibrils in brain tissue has been associated with disease (i.e. Alzheimer's). Recently, however, new evidence has implicated dissolved oligomers or precursors to fibrils in the form of circular annular structures as the possible toxic agents. As a result, there is a great need to study the thermodynamic, kinetic and toxic properties of the oligomer-fiber transition. We chose insulin as a model amyloid protein for this *in vitro* study because it is an amyloid protein, it forms fibrils in ~ 3 h at pH 1.6 and 65°C , exhibits the usual sigmoidal fibril growth curve, has been widely used by others as a model amyloid protein, is freely available and offers little health danger.

Here we probe the reaction scheme and the so-called nucleation process for converting a native folded protein to a beta-sheet rich amyloid fibril. Several different techniques are used to investigate this reaction process including small angle neutron and X-ray scattering, sucrose gradient ultracentrifugation and electron microscopy, and the influence of dissolved osmolytes and the surface chemistry of solid substrates. Cooling and seeding during the lag phase prior to the onset of fibril formation is also very instructive. A simple structural model for the formation of the nucleus is presented. Also, a mechanistic reaction model that simulates the phenomena by incorporating the physical chemistry of nucleation and growth dynamics is presented. Using model fits of the experimental data, the rate constants and Gibbs free energy for nucleation are estimated. Taken all together, the results provide a thermodynamic basis for the mechanism of amyloid fibrillation, offer insight into the role of sugar-based excipients for pharmaceutical formulations and the influence of substrate surface chemistry.