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## AFM Force Spectroscopy: A Novel Tool for the Study of Protein Misfolding Diseases

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### **Background**

Misfolding and aggregation of proteins is a common theme linking a number of neurodegenerative diseases such as Alzheimer's, Huntington's and Parkinson's. Protein misfolding is the very first link in this long chain of events eventually leading to neurodegeneration. Therefore, availability of methods capable of detecting the disease prone protein conformations facilitates the development of novel tools for diagnostic and treating the diseases at the very early stages of development.

### **Goals**

The structure of individual protein molecules within well ordered aggregates can be partially elucidated by traditional structural techniques, including X-ray crystallography, NMR, circular dichroism, fluorescence and IR spectroscopies. However, none of these techniques is fully capable of sensing the misfolded conformation of the protein prior to aggregation. Apparently, the conformation of misfolded protein preceding aggregation differs from that in aggregates, but to what extent it is being different is not clear. High-resolution methods such as x-ray crystallography, NMR, electron microscopy, and atomic force microscopy (AFM) have provided useful data regarding the secondary structure of proteins in nano-assemblies and the morphologies of self-assembled aggregates. However, we still lack of a mechanistic understanding of the process leading to the misfolded conformations of a protein. To address this question, we need to utilize new techniques capable of probing transient conformations of single protein molecules.

### **Approach**

Our central hypothesis is that the transitions of proteins in misfolded states are characterized by elevated interprotein interactions that can be detected by AFM force spectroscopy approach. We developed nanoprobng approach for detection and analyses of transient states based on the fact that misfolded protein conformations differ from folded and other protein conformations by their increased propensity to interact with each other.

### **Summary of findings**

This presentation summarizes our results on the development nanoimaging based approaches for detection and analysis of protein misfolding states tested on a number of proteins and illustrated by  $\alpha$ -synuclein misfolding studies. We used AFM force spectroscopy operating in the single molecule mode to measure for the first time the stability of the a-synuclein dimer, the very first step of the protein aggregation process. The enormously high stability of the misfolded dimer is unexpected finding that provides sheds a new light into the mechanisms of the self-assembly of misfolded proteins into the disease related aggregates.