and others form parallel β-sheet, but the conditions for the preferential formation of one over the other are unknown. Distinct splitting patterns within the amide I' band make FTIR a useful technique for distinguishing between antiparallel and parallel β-sheet (3). In this study, FTIR shows that H1 can also form parallel ß-sheet; H1 is thermostable in both forms (4). The A117L mutant also forms both ß-sheet organizations. Antiparallel A117L aggregates fully dissociate upon heating; the parallel configuration confers thermostability (4). The conversion from antiparallel to parallel ß-sheet is seen only for samples of high peptide concentration and is thermodynamically irreversible. We have proposed a high-concentration-dependent mechanism for the formation of parallel β-sheet aggregates and a structural model of fibril organization that accounts for their thermostability.

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Probing the effect of Heat Shock Protein 70 on the aggregation of α-Synuclein

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The aggregation of α-Synuclein (αS) is crucial to the onset and progression of Parkinson's disease (PD). Recent studies on PD have demonstrated that while αS ultimately forms large dense intracellular plaques (Lewy bodies), the early oligomeric species contribute significantly to cell toxicity. In specific, molecular chaperones such as Heat Shock Protein 70 (HSP-70) have been shown to alter the aggregation properties of αS and are hypothesized to preferentially attack sub-populations of αS oligomers.

Traditionally, fluorescence correlation spectroscopy (FCS) is intended for the study of a narrow population of small diffusing molecules since the intensity fluctuations are proportional to the number of fluorophores and the speed at which they traverse the focal volume. With a solution of unknown size distribution, such as with αS oligomers, the presence of a few large species distorts the autocorrelation curve to a greater degree than the small species thereby impairing our ability to monitor changes in aggregate size. Thus we implemented a new technique, which segments out the large fluctuations and bins them in a burst histogram and autocorrelates the remaining background fluctuations. This technique allows for the concurrent quantification of the distribution of both small and large particles.

As a result of these new findings, we designed a flow chamber that permitted FCS measurements of cytosolic extracts taken from cells co-expressing αS and HSP-70. The presence of large bursts of photons confirmed that over time αS aggregates increase in size and quantity. Co-expression of HSP-70 significantly decreased the number of large aggregates in comparison to cells only expressing αS.

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The Effect of Cations on α-Synuclein Misfolding: Single Molecule AFM Force Spectroscopy Study

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Misfolding and aggregation of α-synuclein are important properties of this intrinsically disordered protein. There is plenty of evidence suggesting that environmental conditions such as metal cations are involved in α-synuclein misfolding and the disease development. Previously, we have applied AFM dynamic force spectroscopy (DFS) to show that at low pH the interaction between α-synucleins increases dramatically and the dimers formed by misfolded α-synuclein have enormously high stability. In this study, we applied the same approach to evaluate the effects of ionic strength and metal cations on α-synuclein misfolding. α-Synuclein was covalently attached to the substrate and probe through C-terminal cysteine. The interaction between α-synucleins was measured in the multiple approach-retraction steps at various locations over the surface. We studied the effect of ionic strength (from 10 mM to 250 mM) on the acidic pH induced α-synuclein misfolding. The DFS study revealed a weak effect of ionic strengths on the majority of DFS parameters. Importantly, the lifetime of the dimers only slightly increases then decreases dependent on ionic strengths and remains in second scale in the ionic strength range. These findings suggest that electrostatic interactions don't play major roles in the α-synuclein misfolding and dimerization. We also studied the effect of metal cations capable of promoting α-synuclein aggregation. We showed that at conditions without significant misfolding of α-synuclein (pH 7.0), the addition of zinc or aluminum cations leads to a dramatic increase of the misfolding events: the probability of events is 7.0% for aluminum and 3.9% for zinc vs. 0.7% in their absence in pH 7.0. Thus, aluminum and zinc cations increase the probability of α-synuclein misfolding explains the role of these cations on the α-synuclein aggregation.

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A Single Mutation in the Non-Amyloidogenic Region of IAPP Greatly Reduces Toxicity

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While the disruption of cellular membranes by prefibrillar states of amyloid proteins is a likely cause of cell-death during amyloid-related diseases, research has been hampered by the complex nature of the aggregation process. The 1-19 fragment of IAPP, a peptide implicated in beta-cell death during type 2 diabetes, is particularly informative for mechanistic studies on amyloid prefibrillar states as it forms toxic oligomers when bound to the membrane but does not progress further to form amyloid fibers. Human $IAPP_{1-19}$ causes a rapid increase in beta-cell islet intracellular calcium levels indicative of a loss of beta-cell membrane integrity. The toxicity of IAPP may be linked to the induction of curvature in the membrane. Solid-state NMR and DSC show that toxic versions of IAPP stabilize negative curvature, while the non-toxic full-length rat IAPP peptide does not. Despite a difference of only one residue from $hIAPP_{1-19}$ (H18R substitution), the rat version of the $IAPP_{1-19}$ peptide is significantly less toxic both *in vitro* and *in vivo*. This difference is reduced at higher peptide to lipid ratios, suggesting that the self-association of $rIAPP_{1-19}$ within the membrane is impaired. The toxicity difference can be traced to the difference in charge at residue 18. At pH 6.0, membrane disruption by $hIAPP_{1-19}$ is significantly reduced and becomes equivalent to that of $rIAPP_{1-19}$. DSC shows that while $hIAPP_{1-19}$ has a minimal effect on the phase transition of lipid vesicles, $rIAPP_{1-19}$ has a strong effect, indicating a surface-associated topology for $rIAPP_{1-19}$ and a transmembrane topology for $hIAPP_{1-19}$; a result in agreement with NMR quenching studies. Our results indicate that the modulation of the peptide orientation in the membrane by His18 plays a key role in the toxicity of hIAPP by altering the interaction with the membrane.

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Curcumin Inhibits The Formation Of Fibrils From Islet Amyloid Polypeptide

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Islet amyloid polypeptide (IAPP) forms assemblies that are toxic to the insulin-producing beta cells found in the pancreas. Inhibiting the formation of the toxic assemblies is therefore an attractive strategy for the development of anti-diabetes drugs. We are studying curcumin, a small molecule that is a component of curry spice, as a potential inhibitor of IAPP aggregation. Our preliminary results obtained by circular dichroism and electron microscopy show that curcumin works best if it is present at an inhibitor: IAPP ratio of 1:1. This suggests that the inhibition occurs at an early stage of the aggregation process. To elucidate the mechanism of inhibition, we are using limited proteolysis monitored by mass spectrometry and two-dimensional ^{1}H NMR spectroscopy. The results of our studies will be presented and discussed.

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High-resolution Structures of Membrane-Bound IAPP Reveal Functional Implications of the Toxicity of Prefibrillar States of Amyloidogenic Proteins

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Disruption of the cellular membrane by the amyloidogenic peptide IAPP (aka amylin) has been implicated in beta-cell death during type 2 diabetes. While the structure of the largely inert fibrillar form of IAPP has been investigated, the structural details of the highly toxic prefibrillar membrane-bound states of IAPP have been elusive. We have shown that a fragment of IAPP (residues 1-19)