

papilloma model. In contrast, the nonnative state showed only a fraction of the proteolytic activity of the native form. This study demonstrates that hexafluoroisopropanol can induce a conformational change in stem bromelain to a form with potentially useful therapeutic properties different from those of the native protein.

### 2308-Pos Board B78

#### Cold Denaturation in a Small Protein Domain

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Small, stable protein domains have become increasingly important as models for protein folding. However, one of the general thermodynamic characteristics of protein structures - cold denaturation - has not been observed for such model domains. We have investigated the thermal unfolding of a small 45 residue  $\alpha$ -helical UBA domain using CD and fluorescence spectroscopy. In addition to a relatively high thermal stability ( $T_m \sim 330\text{K}$ ), we have also detected unfolding at cold temperatures, whose onset begins around 285K in the absence of denaturant. To further probe the cold denaturation, urea was used to destabilize the protein and therefore shift the onset of the cold denaturation to higher temperatures. All experimental data could be explained using a simple thermodynamic model, which assumes linear dependence of the unfolding free energy ( $\Delta G$ ) on the denaturant concentration. The model yields a large positive heat capacity change upon unfolding, which is traditionally associated with solvent exposure of hydrophobic groups. This small UBA domain therefore provides a valuable model for studying the still controversial phenomenon of cold denaturation and for understanding folding of larger proteins, which exhibit cold denaturation behavior.

### 2309-Pos Board B79

#### Perturbing the Central Hydrophobic Cluster (CHC) of A $\beta$ (10-35) by Incorporation of Fluorinated Phenylalanine Derivatives

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One of the putative causes of Alzheimer's disease involves aggregation of misfolded amyloid  $\beta$  (A $\beta$ ), a 39-42 residue polypeptide chain, and its subsequent deposition as amyloid plaques. The aggregation process proceeds via a nucleated polymerization mechanism where disordered peptide monomers interact with each other through hydrophobic interactions and rapidly extend and aggregate to eventually form larger fibrils with a highly ordered cross-strand  $\beta$ -sheet structure. It has also been suggested that the aromatic amino acid residues, tyrosine Y10 and phenylalanines (F19 and F20) in the central hydrophobic cluster (CHC) of the peptide play an important role in fibril assembly. In particular, F19 and F20 are suspected to be the drivers of the aggregation mechanism because of their hydrophobicity and aromaticity. In this context perturbation of the CHC through the introduction of non-natural (fluorinated) amino acids is expected to affect the aggregation process. Fluorinated amino acids in particular demonstrate distinct properties dictated by the presence of highly electronegative and hydrophobic fluorine atoms. However such fluorination is known to potentially eliminate the favorable interaction of aromatic hydrogens with the  $\pi$ -electron cloud, which can affect protein-protein interactions. In the present study the introduction of a pentafluoro-Phe in the hydrophobic core of the 26 residue A $\beta$  peptide (A $\beta$ 10-35) and its effect on fibril formation has been investigated using circular dichroism (CD) and fluorescence methods which indicate a sequential conformational transition of the peptide from random coil  $\rightarrow$  antiparallel  $\beta$ -sheets  $\rightarrow$  parallel  $\beta$ -sheets. Transition time points have been obtained from these methods and compared to those obtained for the non-fluorinated peptide. UV resonance Raman (UVRR) studies have been performed to probe and characterize the vibrational modes of the fluoro-phenylalanines in the peptide and to explore their effect on the Phe-Phe  $\pi$ -stacking interactions.

### 2310-Pos Board B80

#### Kinetic Studies of the Monellin: Evidence for Switching Between Alternative Parallel Pathways

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To determine whether or not a protein uses multiple pathways to fold is an important goal in protein folding studies. When multiple pathways are present, defined by transition states that differ in their compactness and structure but not significantly in energy, they may manifest themselves by causing the dependence on denaturant concentration of the logarithm of the observed rate constant of folding, to have an upward curvature. Upward curvatures are normally not observable, but may become evident upon mutation if the mutation differentially destabilizes the transition states on the parallel pathways. Folding and unfolding kinetic studies performed with heterodimeric monellin

(dcMN) and monomeric monellin (scMN), respectively, using the intrinsic tryptophan fluorescence of the protein as the probe, show chevron arms with upward curvatures. In this study, the folding mechanism of dcMN has been studied over a range of protein and guanidine hydrochloride (GdnHCl) concentrations. Folding is shown to occur in multiple kinetic phases. In the first stage of folding, which is silent to any change in intrinsic fluorescence, the two chains of monellin bind to one another to form an encounter complex. Interrupted folding experiments show that the initial encounter complex folds to native dcMN via two folding routes, and a productive folding intermediate is identified on one but not on both of these routes. The formation of the intermediate occurs in a fast kinetic phase, and its folding to native dcMN occurs in a slow kinetic phase. The folding chevron arms for both the fast and slow phases of folding are shown to have upward curvatures, suggesting that at least two pathways are operational during these kinetic phases of structure formation, and that folding switches from one pathway to the other as the GdnHCl concentration is increased.

### 2311-Pos Board B81

#### Direct Observation of Multistate Folding in a Single Beta-Helical Protein

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Filamentous haemagglutinin (FHA) is the major adhesin of *B. pertussis*, the bacterium that causes whooping cough. It is the prototypical member of the Two-Partner Secretion pathway family, a class of proteins associated with virulence in Gram-negative bacteria. Such proteins are large yet efficiently exported across the bacterial outer membrane without an obvious energy source, suggesting the hypothesis that translocation is driven by folding. Here, we use magnetic tweezers to apply stable and constant forces to single molecules corresponding to the N-terminal 480 amino acids of FHA (which initiate outer membrane translocation) and observe equilibrium unfolding and refolding in multiple discrete steps. This distributed (rather than cooperative) folding of isolated FHA provides evidence for processive, vectorial folding in vivo.

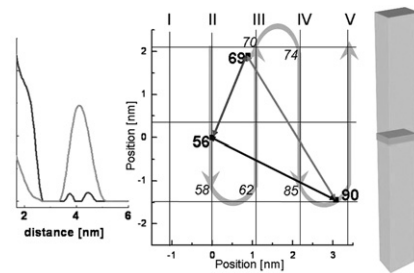
### 2312-Pos Board B82

#### Elucidating the Alpha-Synuclein Fibril Fold by Pulsed EPR

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Amyloid fibrils are constituents of the plaques that are the hallmarks of neurodegenerative diseases. In Parkinson's disease, these plaques (Lewy bodies) consist predominantly of the  $\alpha$ -synuclein ( $\alpha$ S) protein. To understand and interfere with aggregation, the structure of the fibrils (right Fig., green) needs to be known. Here we study the molecular architecture of the fibrils of  $\alpha$ S by measuring distances between pairs of residues in the protein using double electron-electron paramagnetic resonance (DEER). Site-specific spin labeling was employed to create nine doubly labeled  $\alpha$ S-variants that were investigated in the fibrillar state. Diamagnetic dilution with wild-type  $\alpha$ S suppressed intermolecular interactions. The intramolecular distances provide constraints for the fold of the protein inside the fibril. Intramolecular distances were unambiguously observed for four pairs (41/69, 56/69, 56/90 and 69/90). Three of these distances (arrows) provide the constraints to suggest a model for the fold between residues 56 and 90 in the fibril (light blue). Assuming that only parallel  $\beta$ -sheets occur (l.-blue arrows), a model of four adjacent  $\beta$ -strands results (II-V), in which the strands comprise of eight to twelve residues each.



### 2313-Pos Board B83

#### Small Molecule Induced Conversion of Toxic Oligomers to Non-Toxic Beta-Sheet-Rich Amyloid Fibrils

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Several lines of evidence indicate that pre-fibrillar assemblies of amyloidogenic proteins such as soluble oligomers or protofibrils rather than mature, end-stage