N-terminal fragments of the mutant huntingtin protein. The internal fibril structure has remained under debate, largely due to the difficulty to elucidate it in any detail. Enabled by magic angle spinning (MAS) solid-state (ss)NMR, we have obtained site-specific structural and motional constraints on misfolded amyloid-like fibrils for polyQ peptides of various lengths as well as N-terminal huntingtin fragments. The latter includes the first ssNMR studies of U-13C,15N-labeled huntingtin exon1. Thus, we have elucidated the location and key structural features of the amyloid core. The ssNMR data reveal the configurations of the glutamines in the amyloid cores of simple polyQ and huntingtin exon1 fibrils to be very similar (despite quite different aggregation behavior). We also obtained direct insights into the non-polyQ segments and show that these ''flanking domains'' not only fall outside the amyloid core, but also retain remarkable dynamics. We characterize the latter by MAS ssNMR, using quantitative residue-specific measurements of relaxation as well as dipolar order parameters. The distribution and relative dynamics of amyloid and non-amyloid domains suggest a mechanism by which the flanking domains may allow huntingtin binding proteins to influence the stability and formation of fibrils. Moreover, these structural data further our understanding of huntingtin's misfolding and aggregation pathways.

**1937-Pos Board B74**

**Initiating Polyglutamine Aggregation — Computational Clarification of the Structural Details**

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Motivation. In many neurodegenerative diseases, such as Alzheimer's, Parkinson's and Huntington's, cell death is associated with protein misfolding and aggregation. In Huntington's and eight other neurodegenerative diseases the aggregation-prone part of the disease protein is polyglutamine (polyQ). Interestingly, most toxic to cells are not the final aggregates, but some unknown structures occurring when aggregation initiates. Their small size and fleeting nature, however, have prohibited direct experimental observation.

Results. We first show that existing experimental data [1] on polyQ aggregation kinetics implies that an on-pathway polyQ dimer has a characteristic lifetime of seconds. We then use this criterion to check with extensive molecular dynamics simulations the feasibility of six speculated structures to be aggregation-initiating. We find that only structures containing beta-hairpins with interdigitated sidechains fulfill the criterion; structures containing steric zippers or alpha- or beta-helices can be excluded.

Discussion. Combining our findings with recent solid state NMR data that suggests steric zippers to better fit the final fibril [2], we suggest a pathway on which aggregation is initiated by interdigitated hairpins, creating a robust template on which steric zippers may fold.

Implications. The relevance of our suggested pathway stem from the relatively benign effects of the final aggregates versus the high toxicity of early soluble species. Therapeutic strategies could be aimed to encourage steric-zipper-like conformers (and thus aggregate maturation) while discouraging -hairpin formation (and thus aggregate emergence).

This work has been published in Ref. [3].

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**1938-Pos Board B75**

**The Role of Structural Dynamics in Determining the Prion Strain Diversity**

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Prion proteins exhibit alternate structural states and are associated with a number of devastating transmissible diseases. Recent discoveries have revealed the emerging functional roles of prions in a wide range of organisms. For instance, the self-perpetuating conformational change coupled with amyloid formation of a yeast prion protein, Sup35p, a translational termination factor in yeast, is responsible for novel [PSI+] prion phenotypes in Saccharomyces cerevisiae. The 253-residue NM domain of Sup35p is an intrinsically disordered segment and is sufficient for [PSI+] prion initiation and propagation. The NM amyloid recapitulates one of the most spectacular phenomena of prions, namely, the strain-diversity. Earlier it has been shown that two well-defined strains of [PSI+] can be created in vitro. The molecular origin of these strains is postulated to involve diverse, yet related, conformational states and supramolecular packing of proteins within the amyloid fibrils. However, the precise structural and dynamical variations between the prion strains and their distinct physiological impacts remain elusive. To elucidate the structural origins of the prion strains, we took advantage of the fact that NM is devoid of tryptophan and created 19 single tryptophan mutants encompassing the entire length of NM. After establishing that these mutants behave similar to wild-type, we recorded a number of steady-state and dynamic fluorescence readouts that revealed the residue-specific dynamics and supramolecular packing within the amyloids responsible for different strains. The structural differences in two prion strains provided unique molecular insight into the differential binding of Hsp104 that is known to govern the strain propagation. The strain-diversity was further elucidated using time-resolved emission spectra that provided intriguing insights into the water relaxation dynamics within the amyloid architecture. Taken together, our results provide important biophysical clues in discerning the prion strain-diversity in a residue-specific manner.

**1939-Pos Board B76**

**Amyloidogenicity of Immunoglobulin Light Chains**

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Systemic light chain amyloidosis (AL) is a rare protein aggregation disease. It usually strikes in the wake of myeloma, which affects plasma cells in the adaptive immune system. During plasma cell development, the immunoglobulin light chain (LC) genes undergo several rearrangements that leave each clone with a unique protein sequence. The produced monoclonal light chains (LC) are deposited as amyloid in AL but not in Multiple Myeloma (MM) patients. We aim to elucidate the biophysical basis of this difference. In both diseases large amounts of soluble LC are secreted into circulation and excreted with urine. Hence we hypothesize that amyloidogenicity depends on the amyloid formation propensity of the individual LC sequences rather than being a result of different LC concentrations being present in both diseases, which may also alter susceptibilities to the green tea phenol Epigallocatechin-3-gallate (EGCG).

To test this hypothesis we used a simple diafiltration approach to isolate LC from AL and MM patients urine, including only cases with albuminuria less than 5% of total proteinuria. We monitored their aggregation under physiological conditions in presence and absence of EGCG over a time course of three weeks in a Thioflavin T assay and compared the aggregate sizes at different time points by semi-denaturing SDS-PAGE and filter retardation assay. We probed stabilities of native and of aggregated LC by Guanidine and thermal denaturation and imaged aggregate morphologies by atomic force microscopy (AFM).

Each individual LC displayed unique characteristic aggregation kinetics. However, there were no systematic differences between proteins from MM and AL patients. EGCG treatment accelerated the formation of large aggregates that are partially stable against SDS denaturation. By determining the sequence of the LC protein via MS/MS we hope to establish a correlation between sequences, aggregation propensities and clinical parameters in AL and MM.

**1940-Pos Board B77**

**Prediction of the Effects of the Val66Met Polymorphism and Adjacent Structured Domains on the Conformational Ensemble of an Intrinsically Disordered Protein, Brain-Derived Neurotrophic Factor**

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The discovery of Intrinsically Disordered Proteins (IDP) has challenged the structure-function paradigm and required new approaches for identifying functional mechanisms of proteins. Disease-associated Single Nucleotide Polymorphisms (SNP) are common in the disordered regions of proteins (>21.7 %), but not much is known about their effect on the conformational ensemble. Brain Derived Neurotropic Factor (BDNF) belongs to the family of neurotrophins, and facilitates neurogenesis in its short (mature) form but apoptosis in its long (pro) form. A common (4% US population) SNP that results in the Val66Met mutation in the disordered N terminus domain of the long form of BDNF (proBDNF) has been associated with various neurological and psychiatric disorders. We previously explored the effect of this SNP on likely conformations of the BDNF prodomain, using large-scale fully atomistic replica exchange molecular dynamics simulations of the disordered

region, and found significant effects of the single point mutation on the global conformational ensemble. In the present study, we investigate the effects of the presence of the ordered region on this conformational ensemble, as well as the role of the SNP on docking of the disordered prodomain to the ordered region, using fully atomistic Hamiltonian Replica Exchange Simulations. These computational investigations complement previous NMR approaches that were restricted to the isolated prodomain, and serve as model calculations for studying the role of adjacent structured regions on conformations of intrinsically disordered regions.

**1941-Pos Board B78**

**Structural Stability of Diabetes-Related Amylin Protofilaments: Applications to Fibril Design**

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We study, using atomistic molecular dynamics and coarse-grained methods, the conformational dynamics and structural stability of amyloid fibrils formed by the Islet Amyloid Polypeptide (IAPP), which is generally known as amylin. Human IAPP (hIAPP) is a co-secretion with insulin and widely found in fibril form in patients suffering with type-2 diabetes. New drugs may be developed if we understand the molecular structures of amylin fibrils, possibly leading to treatments to prevent the fibril aggregation. We build atomistic models of hIAPP amyloid protofilaments that are in agreement with previously published solid state NMR data. Our study includes different conformations and fibril topologies, and tests the effect of mutated sequences, including naturally occurring ones, that can alter the fibril stability. In particular we identify new mutations that can lead to new fibril types and compare their conformational properties. A special case is considered for amylin from human and rat organisms. In spite of relatively small sequence differences, the rodent amylin does not aggregate into fibrils, making it an excellent test case.

**1942-Pos Board B79**

**Explosive Fibrillation Kinetics of Two-Chain Insulin Fragment Released upon Partial Digestion with Pepsin**

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Proteases are recognized for their role in the emergence of highly aggregation-prone protein fragments in vivo. On the other hand, limited proteolysis in vitro is often used to probe different phases of amyloidogenic pathways. Here we show that moderate amounts of pepsin induce ''explosive'' fibrillation in acidified samples of bovine insulin. Biochemical analysis of the pepsin-induced fibrils reveals previously unreported two-chain peptide with potent amyloidogenic properties as the main building block. The peptide (named 'H') comprises of N-terminal fragments of insulin A- and B-chains linked by disulfide bond between Cys7A-Cys7B and conceals up to 8 additional pepsin-cleavage sites which become protected upon fast fibrillation unless concentration of the enzyme is increased leading to complete digestion of insulin. Fibrils built of H-peptides are similar in terms of morphology (as probed by AFM) and infrared features to typical bovine insulin fibrils, but they appear to lack the ability to seed fibrillation of intact insulin. Controlled re-association of these fragments leads to 'explosive' fibrillation only under non-reducing conditions implying the key role of the disulfide bonds in the amyloidogenicity of H-peptides.

Our study highlights the role of dynamics of the disulfide-bonded N-terminal fragments of A- and B-chains in insulin amyloidogenesis.

**1943-Pos Board B80**

**Elucidating the Role of Oligomers in Insulin Aggregation using Biophysical Methods**

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Protein misfolding and aberrant fibrillization underlie many neurodegenerative conditions, such as Alzheimer's and Parkinson's disease. Insulin, which is composed of two covalently bonded peptide chains, exists in vivo mostly in a native hexameric state but becomes amyloidogenic under certain conditions: at high temperature with neutral pH (7.4) and agitation or with low pH (1.6) and quiescence. To investigate the mechanisms that drive insulin aggregation, we monitor its self-assembly into fibrils by kinetic fluorescence spectroscopy, which uses Thioflavin T (ThT), a fluorescent dye that binds to the cross-β structure of amyloid fibrils. At low pH, insulin behaves similarly to other amyloid proteins; kinetic rate of fibrillization increases with concentration. At neutral pH, we observe an increase of the kinetic rate of fibrillization with low insulin concentration (2.5 – 25 μM), whereas at higher concentrations (25 – 100 μM) the opposite trend is observed. To explain this observation, we utilize photo induced cross-linking of unmodified proteins (PICUP) and Sodium Dodecyl Sulfate-Polyacrylamide gel electrophoresis (SDS-PAGE) to determine the oligomeric population of pre-fibrillar stages of insulin self-assembly. Preliminary results show a shift toward larger oligomers at insulin concentrations in the vicinity of 25 μM. As self-assembly advances and fibrils start to form (as observed by ThT fluorescence), PICUP/SDS-PAGE shows progressively decreased oligomer abundances. Insulin aggregation is also monitored via atomic force microscopy (AFM) to investigate differences in morphology between the two methods used to induce aggregation and the corresponding time evolution of oligomeric species. Our results are consistent with oligomer formation that is on the pathway to fibril formation, thereby elucidating a key interplay between oligomers and fibrils in insulin aggregation.

**1944-Pos Board B81**

**DMSO Induced Breaking up of Insulin Fibrils Monitored by Vibrational Circular Dichroism**

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Bovine insulin can form stable β-sheet-rich amyloid aggregations composing of several protofibrils and adopt variable morphologies based on the fibrillation condition. Vibrational circular dichroism (VCD) was reported as a very useful probe for charactering the chirality of amyloid aggregates and detecting formation of extended, twisted fibrils.(1) We studied the effect of adding dimethyl sulfoxide (DMSO) to aqueous insulin fibrils and monitored their destabilization by VCD. We compared two types of insulin fibrils depending on sample preparation protocol, one type can have oppositely signed induced circular dichroism for amyloid-bound THT,(2) and the other type has oppositely signed VCD.(1) Transmission electron microscopy (TEM) was used to correlate the morphology with VCD spectrum to show both the molecular morphology and supramolecular chirality aspects of the DMSO induced insulin fibril breaking-up process. The two types of insulin fibrils behaved differently on addition of DMSO, but both of them were eventually denatured by high concentrated DMSO.

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**1945-Pos Board B82**

**The Intrinsically Disordered Termini of zDHHC S-Palmitoyltransferases Facilitate Multiple Regulatory Functions**

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zDHHC protein acyltransferases (PATs) are a family of membrane proteins that catalyze the reversible post-translational lipidation known as palmitoylation, a process essential to normal cellular function through facilitation of membrane attachment, subcellular trafficking, and protein stability. While transmembrane proteins such as PATs are mostly ordered due to the hydrophobic membrane environment, they have cytoplasmic tails which tend to lack stable three-dimensional structure. The aim of this study was to understand the structural and functional implications of disordered PAT termini using computational, biochemical, and biophysical approaches. Intrinsic disorder prediction indicates that a conserved α-helical molecular recognition feature (MoRF) exists in the C-termini of all PATs. In the human and yeast Ras PATs (zDHHC9 and Erf2, respectively), this region was found to be essential to palmitoyltransferase function in vivo and in vitro. Additional experiments suggest that the MoRF participates in previously undescribed protein-protein interactions. The disordered termini of Erf2 also facilitate multiple regulatory post-translational modifications including phosphorylation, acetylation, and ubiquitination. As PTMs and protein-protein interactions of PATs have been poorly described, elucidation of the structure-function relationships and modifications of intrinsically disordered regions in PATs potentially represents a novel paradigm of pharmacological interrogation of protein palmitoylation.