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

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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 con-

centration. At neutral pH, we observe an increase of the kinetic rate of fibrillization with low insulin concentration (2.5 – 25 μ M), whereas at higher concentrations (25 – 100 μ M) 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 μ M. 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.

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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 characterizing 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.