Disease (CMTD), the most common disorder of the PNS. Disease mutations induce misassembly of PMP22, resulting both in loss of its function and toxic accumulation of misfolded PMP22 in the cell. Here we present a structural comparison of the wild type and the L16P disease-linked mutant form of human PMP22 to obtain insight into the molecular basis of CMTD. Human PMP22 was expressed in *Escherichia coli*, and purified in the detergent tetradecylphosphocholine. The purified protein provided moderately well dispersed ^{1}H-^{15}N TROSY spectra. NMR resonance assignments for the wild type protein revealed that the 72 observed backbone amide peaks out of 157 expected originate exclusively from the N-terminal STREP tag, transmembrane region 1 (TM1), extracellular loop 1 (ECL1), and the extreme C-terminus. Chemical shift index analysis suggested the residues from TM1 (Met1 to Ile29) form an α-helix, while no secondary structure was predicted for ECL1 (Val30 to Pro58). The L16P mutant was analyzed in a similar manner. A significant finding for the mutant was that the resonances from Ile8 to Val17 located at the middle of TM1 were not observed due to line broadening. Moreover, chemical shift perturbations were observed for residues from Leu18 to Ile24 which are located at the C-terminal end of TM1. These observations suggest that the L16P mutation induced a global conformational change in TM1 that results in its recognition as being folding-defective by components of membrane protein folding quality control system of the endoplasmic reticulum.

Protein Aggregates III

3375-Pos

Possible Pathway between Alpha Helical and Beta Helical Structures of the C-terminal in the Mammalian Prion Protein

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The normal form of the prion protein(PrP^{C}) has mostly alpha-helical (AH) secondary structure in the C-terminal region (residues 166-230), while the infectious form (PrP^{Sc}) has been proposed to have a left-handed beta helical (LHBH) structure(1). The mechanism of conformational change from PrP^{C} to PrP^{Sc} is unknown, but recent electron microscope data(2) and computer modeling(3) of in vitro grown prion fibrils suggest a possible LHBH structure in the C-terminal region. We use high temperature (500K) AMBER molecular dynamics over 10 ns runtimes to study the unfolding transitions commencing from both LHBH and AH C-terminal starting structures. Using stability, contact map, and energetic analyses we find that both structures unfold to very similar AH-like conformations and discuss the potential implications of this result for normal prion cellular function and for prion disease.

References

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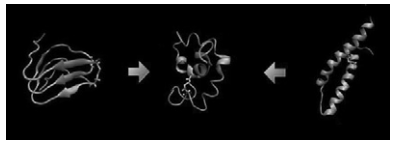
3) Kunes, K. C., et al. (2008) *Prion* **2,** 81-90.

The image below compares computational models of the initial LHBH structure(left), the initial AH structure (right), and the unfolded AH-like structure(middle).



3376-Pos

Molecular Mechanism of Inhibition of Amyloid Formation by Inositol

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Alzheimer's disease (AD) is a severe neurodegenerative disease with no cure. Currently, one method of targeting the underlying disease is to prevent or reverse the amyloid formation of Abeta1-42, a key pathological hallmark of AD. Scyllo-inositol is a promising small-molecule therapeutic that is found to exhibit stereochemistry dependent inhibition of formation of Abeta fibrils in vitro and is currently in phase II of clinical trials. However, the mechanism of action of scyllo-inositol at the molecular level is not known. We perform extensive atomistic molecular dynamics simulations of scyllo-inositol and its inactive stereoisomer, chiro-inositol, to systematically compare and characterize both the binding mode and the effect of inositol on the structure, morphology and aggregation equilibrium of the amyloidogenic fragment of Abeta42, KLVFFAE (Abeta16-22).

3377-Pos

Side Chain Interactions can Impede Amyloid Fibril Growth:Replica Exchange Simulations of Abeta Peptide Mutant

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Aggregation of Aβ peptides is related to the onset of Alzheimer's disease, but the molecular mechanism of the Aβ fibril formation is still poorly understood. Recently, we have studied the thermodynamics and free energy landscape of Aβ fibril growth using a hexamer system of $A\beta_{10\text{-}40}$ peptides by replica exchange molecular dynamics simulations and atomistic implicit solvent model. The system consisted of four peptides forming a fibril fragment and two incoming peptides binding to the fibril edge. We have demonstrated that deposition of the peptides onto the fibril follows the ''dock-lock'' mechanism. In the docking stage, disordered peptides dock to the fibril without their incorporation into the ordered fibril structure. In the locking stage, the incoming peptides form parallel β-sheets with the fibril. In this presentation, we report the effect of D23Y mutation in $A\beta_{10\text{-}40}$ peptides focusing on the changes in the deposition free energy landscape and in the interactions between incoming peptides and the fibril. We found that although D23Y mutation has a weak impact on the docking stage, it induces strong stabilizing effect on the locking stage of fibril growth. We explain these findings by elimination of off-registry side-chain interactions formed by Asp23 in the wild-type Aβ sequence. We conclude that strong off-registry side chain interactions have a capacity to impede fibril growth.

3378-Pos

Elucidating the Association and Dissociation Mechanism of β-Amyloid Protein by Targeted Molecular Dynamics Simulations

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The amyloid-β (Aβ) proteins are responsible for amyloid plaques in Alzheimer's disease and have been the most widely studied subject in the process of fibril growth. Although much progress has been made to elucidate amyloid fibril properties at a molecular level, the full identification and characterization of all the conformational states and oligomeric structures in the aggregation process and all the conformational changes that link between those different states are still needed to be revealed. Here, we present the results of targeted molecular dynamics (TMD) simulations with explicit water to investigate the structural and mechanistic aspects of the association and the dissociation of the Aβ42 dimer. We will discuss the reversibility and the driving forces of the Aβ42 dimerization process with several order parameters along the protein aggregation pathway.

3379-Pos

Charactering Amyloid-Beta Protein Misfolding from Molecular Dynamics Simulation with Explicit Water

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Formation of amyloid-beta (Aß) protein aggregates is the primary cause of amyloid diseases including Alzheimer's disease (AD). Here, we present the state-of-the-art atomic-level characterization of the misfolded state of Aß42 and early misfolding events from helical structure to form aggregation-prone structure in water by using all-atom molecular dynamics (MD) simulations in explicit water environment. Our simulations reveal one of the most important yet unsolved structural mysteries in early misfolding steps that the aggregation-prone structure (APS) of Aß42 is characterized by the non-helical backbone H-bond formation between K16L17 and V39V40I41 associated with the expansion of the hydrophobic exposure. Characterizing the nature of the misfolded state (APS) of Aß42, we provide new insight into the experimentally observed different aggregation propensities of Aß42 compared to those of Aß40. Based on the structural features of APS, we also speculated the hypothetical aggregation mechanism from APS of Aß42 to form fibril accounting three mandatory steps. The structural and mechanistic observations based on these simulations agree with the recent NMR experiments and provide the driving force and structural origin for the Aß42 aggregation process to cause AD.

3380-Pos

Fiber Formation of Silk-Like Proteins

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Beta-sheet forming proteins can fold and assemble into long fibers that play a structural role. While this phenomenon is most famous for its role in amyloidogenic diseases, such fibers also have high potential as biomaterials. In both cases, it is crucial to understand the entire formation process, but the fact that folding and assembly are often intertwined makes this very difficult.
The natural silk fibroin consists of several ''crystalline'' domains with a highly repetitive amino acid sequence, linked through hydrophilic, amorphous spacer sequences. Here, we focus on an artificial silk protein with a (EGAGAGA)x repeat for the crystalline domain (E is glutamate, G is glycine, A is alanine; x denotes the number of repeats) with hydrophilic flanking sequences. Experiments have shown that upon a change in pH, the EGAGAGA repeat will fold and

aggregate whereas the hydrophilic flanks prevent random aggregation and drive the system to form fibers [1,2].
Based on our atomistic simulations of the (EGAGAGA)x repeat and the hydrophilic sequences seperately, we have developed a coarse grained protein model that allows us to study fiber formation as well as certain characteristics of the mature fibers [3]. Although our model has been developed for the artificial silk protein it can also be applied for natural occurring proteins such as amyloids and may be extended to study other fiber forming proteins.
[1] Smeenk et al., Angew. Chem. Int. Ed., 2005, 44, 1968-1971
[2] Martens et al., Macromolecules, 2009, 42, 1002-1009.
[3] Schor et al. Faraday Discuss., 2010, DOI: 10.1039/b901608b

3381-Pos
The Spontaneous Aggregation of Steric Zipper Peptides Studied by Molecular Dynamics Simulations
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Recently obtained crystal structures of truncated fragments of proteins provide detailed structural insights into beta-sheet rich aggregates, known as amyloid fibrils [1,2]. The arrangement of these short model peptides revealed a common steric zipper motif in the crystalline state. Two sheets of peptide strands are interfaced by a dry and tight zipper structure with a high degree of sidechain complementarity. Combined experimental data suggests that steric zippers may represent a general feature of amyloid formation. However, a thorough understanding of the aggregation process and the structural characterization of its multitude of conformational states is still lacking.
We employ molecular dynamics simulations in an explicit solvent environment to study biomolecular aggregation at atomistic detail with the aim to unveil the energetic and structural determinants that drive the formation of amyloidogenic peptide assemblies and also stabilize the formed aggregates.
Starting from separated peptide chains with random conformations, we monitor the primary events of aggregation and find a rapid clustering of the peptides accompanied by an increased number of inter-molecular hydrogen bonds and the spontaneous formation of beta-sheet rich oligomers. Some of the peptide aggregates feature structural characteristics of the crystalline conformation (e.g. beta-sheet bilayers with dry interface), but also interconvert with conformationally distinct oligomeric states.
By mapping the conformational ensembles we were able to describe the different topologies of the system, which helps to yield insight into possible common mechanistic steps found along the aggregation pathway. The goal of our work is to fully characterize the aggregation behaviour of small model peptides and test our findings with results from /in vitro/ experiments (EM, NMR) with a particular focus on aggregation-prone sequences of tau, insulin and alpha-synuclein.
[1] Nelson et al., Nature, 2005
[2] Sawaya et al., Nature, 2007

3382-Pos
Beta-Barrel Hypothesis: Structural Insights to Oligomeric Prion Conformation
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The denaturation of prion protein (PrP) and the concurrent formation of beta-sheet rich isoform has been accredited to the etiology of the prion diseases. Accumulating biochemical evidences underline the critical role of oligomeric PrP conformations emerging from the early stage of the denaturation process. However detailed structural information on the oligomeric isoforms remains elusive, which hampers precise description of the pathological process. Recently we proposed a new structural hypothesis for the oligomeric PrP species comprised of a short PrP construct (Human PrP 175-217) based on experimental findings. We postulated that 1) monomers adopt beta-hairpin conformation and 2) assemble as beta-barrel quaternary structure. These assumptions provided a comprehensive explanation for the experimental findings suggesting beta-sheet rich structure including circular dichroism (CD) spectrum and Fourier transformed infra-red spectroscopy (FTIR) as well as the presence of disulfide bridge between CYS-179 and CYS-214. To be more specific, we constructed various beta-barrel models differing in number of monomers, intermolecular hydrogen bond pattern and side-chains facing exterior of the barrel. Those models were refined extensively using a protein structure prediction tool (Rosetta). Structural energy profile of the predicted oligomer models was consistently lower than that of native like monomer or oligomers comprised of partially denatured monomers. Also the smallest stable oligomer was predicted to be an octamer, which is in good agreement with available mass spectrometric data. Finally, we discussed a possible generality between protein denaturation and amyloidogenesis problems in general, by comparing our model with a oligomeric assembly model for the pathological amyloid beta protein (Abeta).

3383-Pos
Polymophism of A-Beta1-42 Peptide Oligomer - Membrane Interactions
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Recently, alternative propositions have been put forward to explain the pathogenesis of Alzheimer's disease with the possibility that amyloid peptides form unregulated pores or ion channels in membranes. In this study, we compared several ion channel aggregation models of with 24 Aβ1-42 peptides in a membrane environment, using Molecular Dynamics simulations. Our results indicated that like in solution, the polymorphism of Aβ1-42 oligomers also relate to possible ion conductance induced by Aβ1-42 peptides.

3384-Pos
Modeling Amyloid Oligomers with Different Structural Morphologies
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The aggregation of monomeric proteins/peptides to form ordered amyloid oligomers/fibrils is a pathogenic feature of many degenerative diseases including Alzheimer's, Parkinson's, and prion diseases. Despite of significant progress, oligomeric structures and associated toxicity at the very early stage of aggregation remain unclear. Structural knowledge of these oligomers is essential for understanding the pathology of amyloidoses and for rationally designing drugs against amyloid diseases. In this work, molecular modeling and simulations are performed to examine the conformational preference and structural characteristics of preformed oligomers with different structural morphologies (micelles, annulars, triangulars, and linears) and amyloid peptides (Abeta, hIAPP, GNNQQNY, and K3). We identify several stable oligomeric structures with different structural morphologies and sequences, delineate several common features in amyloid structures, and illustrate aggregation driving forces that stabilize these oligomeric structures. Structural comparison among different oligomers suggests that the aggregation mechanism leading to distinct morphologies and the aggregation pathways is sequence specific, due to differences in side-chain packing arrangements, intermolecular driving forces, sequence composition, and residue positions. Moreover, we are also modeling the stable A-beta oligomers on the lipid bilayers to illustrate the postulated mechanism of membrane damage associated with amyloid toxicity.

3385-Pos
A Single-Molecule Approach to Tau
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Tau is a protein associated with bundles of microtubules, while tau/tau interactions can lead to aggregates thought to underlie Alzheimer's disease. Here, we investigate the utility of a multiplexed single-molecule manipulation approach to give information on tau structure and tau/tau interactions: Previously we demonstrated the ability to perform several single molecule measurements in parallel in a multiplexed magnetic tweezers assay (Rev. Sci. Instrum. 79, 094301 (2008)), enhancing the statistical significance of the data. For testing the capability of this tool in protein folding studies, we present data on nucleic acid hairpins as a model system. We directly observe high resolution hairpin opening and closing events on several single molecule tethers simultaneously subject to the same critical force. We then describe experiments to observe the thermodynamics and kinetics of protein aggregation by i) immobilizing and studying tau in isolation then ii) studying interactions between immobilized tau with tau free in solution.

3386-Pos
Pre-Amyloid States of Islet Amyloid Polypeptide Examined by Single-Particle Fluorescence
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Islet amyloid polypeptide (IAPP or amylin) is a peptide hormone cosecreted with insulin by the pancreas that displays potent amyloidogenic activity. In vitro studies demonstrate that IAPP is capable of disrupting lipid bilayers, suggesting a possible mechanism for IAPP-induced beta-cell death in Type II Diabetes Mellitus. Of particular interest are oligomeric IAPP species, which are believed to mediate membrane leakage, as well as to be intermediates in amyloid formation. IAPP oligomers are likely to be transient and heterogeneous, and so a detailed dynamic and functional characterization of these critical structures has been challenging. We have used single-molecule Förster resonance energy transfer (FRET) to study IAPP conformations in solution; bound to model membranes; and in the presence of insulin, which exerts