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

a cytoprotective effect. Intermolecular FRET was used to characterize the topology, packing and dimensions of IAPP oligomers. FRET measurements provided experimental constraints for atomistic modeling and MD simulations both of IAPP monomers and oligomers. Simultaneously, we have used fluorescence correlation spectroscopy (FCS) to explore the interactions of IAPP with lipid bilayers, probing the thermodynamic landscape of membrane-catalyzed IAPP oligomer assembly. Single-molecule techniques provide unique experimental insight into the processes governing the membrane binding and aggregation of IAPP and similar amyloidogenic peptides.

3387-Pos

N&B and Cross-N&B Analysis Detect Oligomerization of Huntingtin in Live Cells

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Aggregation of misfolded proteins is a hallmark of several neurodegenerative diseases such as Huntington's disease (HD). HD is caused by a mutation of Huntingtin caused by an elongation of a polyglutamine (polyQ) sequence in the protein. Here we describe the application of the recently developed Number and molecular Brightness method (N&B) to monitor the aggregation process of Huntingtin exon1 (Httex1). N&B measures the molecular brightness of the protein aggregates in the entire cell non-invasively based on the fluctuation dynamics at each pixel of an image. This analysis provides a map of aggregation with pixel resolution.

We observed the behavior of Httex1-97QP-EGFP this is a construct with 97 polyQ repeats corresponding to Juvenile onset of the disease.

We preformed experiments in ST14A cells transfected with Httex1-97QP-EGFP. We establish that the process of nucleation leading to inclusion formation has four phases: i) Initially only monomers are present; ii) Following an increase in protein concentration (~1 μM), due to protein accumulation, small oligomers (8-15 proteins) form throughout the cell; iii) At higher protein concentrations, an inclusion is formed in the cytoplasm; iv) The inclusion recruits most of the Httex1 protein in the cell, including those in the nucleus, leaving only monomers at very low concentration.

We also performed cross-N&B analysis to measure the size of the oligomeric species. Cross-N&B recovers the stoichiometry of the complexes from the simultaneous fluctuations of the fluorescence intensity in two image channels. The experiments were done on ST14A cells co-transfected with Httex1-97QP-EGFP and Httex1-97QP-mCherry. These experiments confirmed the mechanism of aggregation observed by N&B and the range of size of the oligomers.

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3388-Pos

Amyloid-β and α-Synuclein Aggregate in Live Cells at Concentrations Far Below their in vitro Solubility Limits

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Amyloid protein aggregates are believed to be the key cause of neurodegenerative diseases such as Alzheimer's and Parkinson's, but the mechanism of their biological action is far from understood. Different studies have implicated different aggregated forms of these proteins as the key toxic elements. Significantly, these studies typically use concentrations in the μM range to get the proteins to aggregate, but this is at least an order of magnitude higher than the concentrations observed *in vivo*. Here we examine the aggregation of amyloid beta (Aβ) and α-synuclein on cell membranes and inside live cells at sub-μM concentrations, using coupled fluorescence correlation spectroscopy, confocal microscopy and time correlated single photon counting techniques. For Aβ, we find that only small aggregates (<6 nm hydrodynamic radius) form at 350 nM concentration *in vitro*, but large aggregates of Aβ are present on the cell membrane, together with much smaller species (monomers or small oligomers). When the *in vitro* concentration is lowered to 150 nM, the solution structures are even smaller (<4 nm, but significantly, still at least dimeric) and no large multimers form on the membrane, though the smaller species are still present. We conclude that Aβ aggregates at much lower concentrations on the membrane, possibly because of the lower free energy of association required for such aggregation in a two dimensional system. For α -synuclein, we find that a 550 nM solution which does not form any aggregates in the extra- cellular medium, develops large aggregates in the cytoplasm. Our results challenge the hypotheses that specific aggregate structures (e.g. donut shaped, spherical or protofibrillar aggregates) that form in the solution phase are responsible for the physiological effects of Aβ or α -synuclein.

3389-Pos

Amyloid Beta Oligomer Studied by Newly Developed Single Molecule Analysis Method

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Amyloid beta (Ab) is 4 kDa peptide which forms aggregates such as oligomers and fibrils. They have been considered to cause Alzheimer's diseases (AD). Recent results have suggested that soluble Ab oligomers are the causative agent of AD since such oligomers are more cytotoxic than fibrils. It was also suggested that Ab oligomers affect not only cell death but also early stage of cell dysfunction and cause memory loss.

However, the formation mechanism of these soluble oligomers is still unknown. In this study, we developed new single molecule analysis method that can analyze Ab oligomer distribution. For this purpose, we combined total internal reflection fluorescence microscopy (TIRFM) with photon counting histogram (PCH) (Terada, N. et al. (2007) Biophys. J. 92, 2162). Using TIRFM, fluorescent intensity of monomer is obtained from discrete photobleaching. Using PCH method, the number of protomers in oligomers and concentrations are obtained from histograms of photons from fluorescent molecules diffusing through the confocal volume.

A model Ab oligomer was prepared by sonication treatment of Ab fibrils made from FITC labeled Ab monomer. The number of Ab monomer in a single Ab oligomer was estimated by TIRFM, which agrees well with the results of PCH analysis. Thus we concluded that PCH can be applied to analyze Ab oligomer formation. Next, Ab oligomers were formed at physiological Ab concentration (LeVineIII, H. (2004) Anal. Biochem. 335, 81). Oligomer formation was observed with TIRFM analysis. PCH analysis is now in pregress.

3390-Pos

Investigating Amyloid Nucleation and Growth with Fluctuation Microscopy

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The nucleation and growth mechanisms in amyloid forming materials are of high interest due to their importance in human diseases and also due to their potential applications as functional nano-materials. While much is known about the β-sheet secondary structure of amyloids, the nucleation mechanism and self-assembly processes remain poorly understood. Multiple intermediate species have been proposed to play important roles in the self assembly process, yet these states remain poorly defined or have not been unambiguously identified in solution. We have used single molecule fluorescence spectroscopy and two-photon fluorescence imaging to investigate early events in amyloid nucleation and growth. Our observations have demonstrated that unstructured protein aggregates play a key role in the earliest phases of self assembly.

3391-Pos

Detecting and Characterizing Amyloid-$\beta_{1\text{-}40}$ Oligomers using Single Molecule Fluorescence

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The devastating symptoms of Alzheimer's Disease (AD) have been attributed to the behavior of aggregated Amyloid-β (Aβ) peptides. Cleaved from the extra-cellular portion of the transmembrane receptor, amyloid precursor protein (APP), Aβ has a very high propensity to aggregate into fibrils with a cross-β-sheet structure. Though fibrillar plaques have been seen as a hallmark of AD, there has recently been increasing evidence suggesting that smaller soluble Aβ oligomers are the agents of neuronal toxicity. Molecular level characterization of these oligomers using conventional biochemical techniques has been very difficult as they are both short-lived and heterogeneous.

In this work we have used a single molecule fluorescence microscopy technique to follow the formation and resolve distributions of these oligomers. Two-color coincidence detection (TCCD) has the ability to detect such transient complexes even when they comprise only 0.1% of the population (*1, 2*). In this work, we have detected and characterized the species ranging from dimers to 50-mers formed during the oligomerization of monomeric $A\beta_{1\text{-}40}$ (the 40-amino acid portion of Aβ) as well as those resulting from breakage of $A\beta_{1\text{-}40}$ fibrils. Through experiments tracking the formation of oligomers throughout the Aβ aggregation process in real-time, we have gained novel insights into the mechanisms of oligomer formation and fibril breakage.

1. A. Orte, R. Clarke, S. Balasubramanian, D. Klenerman, *Anal. Chem.* **78**, 7707 (2006).
2. A. Orte *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* **105**, 14424 (2008).