**1366-Pos Board B96**

**Single Molecule FRET Characterization of Oligomers from Alpha-Synuclein Early Onset Parkinson's Disease Mutants**

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Parkinson's disease is the most diffused neurodegenerative disease involving movements. The protein alpha-synuclein must be linked to the disease by two lines of evidence: first, amyloid-like fibrils of the protein are found in patients' brains; second, three missense mutations in the gene of alpha-synuclein and the gene triplication itself cause autosomal dominant early onset forms of the disease. Recent outcomes underlined that alpha-synuclein oligomers are toxic for cells and may be responsible for cell death in Parkinson's disease. The precise role of oligomers in the etiopathogenesis of the disease is still under debate. In this study we use single molecule FRET to catch differences in the overall structure of oligomers generated from wild-type and A53T, A30P and E46K mutants. FRET efficiencies distributions reported by the mutants oligomers sets are different compared to the wild-type protein. This opens the possibility that oligomers with different structures may have different targets or may be differently processed by cells. Results obtained with this technique may contribute to the understanding of early onset Parkinson's disease generated by the mutants.

**1367-Pos Board B97**

**Long-Range Distance Constraints for the Fibril Fold of Parkinson's Protein Alpha-Synuclein**

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Sequence-specific information on the fibril-fold of α-synuclein (αS), which is required for understanding fibrilization and the role of αS in Parkinson's disease, is still scarce. Double Electron Electron Resonance (DEER) on doubly spin-labelled αS potentially gives access to distances in the nm-range. Fibrillizing the labelled protein in a background of wild-type αS, intramolecular distances are emphasized, but intermolecular distances cannot be suppressed completely, leading to superposition traces that are difficult to disentangle. Nevertheless, long-range distance constraints are beginning to emerge. We compare distances between residue pairs 56-69, 56-90 and 69-90[1] to recent results of Pornsuwan et al.[2]

Are these constraints consistent with the simplest model, in which the protein is in a plane perpendicular to the fibril axis, with all β-sheets parallel? If not, it could be that a more complicated fold pertains, such as a situation where not all the β-sheets are parallel to each other or that the protein extends over different planes along the fibril axis. Also, different fibril forms, e.g. different morphologies, could be the cause. We will discuss these possibilities.

*References*

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[2]Kayagina et al. Biophys. J. (2011) 101, L1-L3.
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**1368-Pos Board B98**

**Structure of the Transient, Membrane-Active Amyloid Beta Oligomers in Physiological Solutions Probed by a Combination of Fluorescence and Solid State NMR**

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Small oligomers of Aβ are suspected to initiate Alzheimer's disease (AD). However, their low concentration and transient nature under physiological conditions have thwarted efforts to determine their structure. Using time resolved FRET spectroscopy, we have recently shown that the monomers are relatively unstructured, and the aggregation process starts with the two terminals of the peptide coming closer in the small oligomers (n-mers with n<10)1. Here we develop a method which combines rapid fluorescence techniques with slower two-dimensional solid state NMR, and probe nascent Aβ40 oligomers which demonstrate an enhanced ability to attach to cell membranes compared to the monomers2. We find that the conformation of the two hydrophobic arms (residues 10-22 and 29-40) have already reached the conformation observed in the fibrils. However, the turn region (residues 23 to 28) and the N-terminal tail (residues 1-9) are strikingly different. Notably, majority of the Aβ mutants linked to familial AD map to these two regions, and toxicity modulators such as $Zn^{++}$ affect the same region3. Our results provide specific structural targets for therapeutic strategies designed to modulate Aβ oligomers.

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**1369-Pos Board B99**

**Systematic Characterization of Wild Type and Familial Alzheimer's Disease Mutant Aβ Monomers Through the Convergence of Ensembles Simulated with Different Force Fields**

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Amyloid β (Aβ) monomers represent the base state in the pathways of aggregation that result in the fibrils and oligomers involved in Alzheimer's disease (AD). The structural properties of these intrinsically disordered peptides remain unclear despite extensive efforts to resolve these through experiment and computation. Comparison of all-atom, explicitly solvated simulations of wild type Aβ monomers simulated with different force fields (OPLS-AA/L and AMBER99sb-ILDN) and water models (TIP3P and TIP4P-Ew) nevertheless demonstrate a convergence in structural properties and good agreement with experimental NMR observables. In Aβ42, antiparallel β-hairpin structure between L17-A21, A30-L34, and V40-I41 is prevalent in these ensembles. While residues 21-30 forms an interceding region in both simulations that rarely interacts with the majority of the protein, the structure of this region and the electrostatic interactions that characterize it are notably different between the two. To further explore these differences, NMR experiments and simulations using both combinations have been conducted for familial AD (FAD) mutations that perturb residues 22 and 23, observed in our wild type Aβ simulation data to be pivotal in determining the structure of this central region. The characterizations made here suggest simulation conditions that best reproduce the experimental data and help clarify how FAD mutations drive Aβ to aberrant aggregation pathways that result in disease phenotypes.

**1370-Pos Board B100**

**Structural and Mechanistic Analyses of the Effects of Small Compounds on Amyloid Beta Self-Assembly**

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Although the natural tendency of proteins is to acquire soluble native-like functional states, they are able to undertake alternative pathways, such as self-association into ordered insoluble amyloid fibrils. The latter are protein aggregates that share common cross-β sheet structures and give rise to various incurable disorders, such as Alzheimer's and Parkinson's diseases. The abnormal self-assembly of amyloid beta (Aβ), an intrinsically disordered peptide, into neurotoxic oligomers and fibrils is strongly associated with Alzheimer's disease, thus prompting a comprehensive search for small compounds capable of inhibiting its aggregation. Although many compounds have been reported with this effect, the mechanisms by which they act are still largely unclear. In the present study, we use Small-Angle X-Ray Scattering (SAXS) as a high-throughput tool to screen an in-house fragment-based library in an attempt to identify new potential inhibitors targeting Aβ aggregation. We follow the fibrillation process of Aβ in solution over time in order to resolve major co-exisiting species and monitor by SAXS the effect of small compounds on these species. This approach is expected to provide molecular insights into Aβ self-assembly mechanism and lead effectively to much needed new treatment options.

**1371-Pos Board B101**

**Decoupling Conformation, Aggregation and Function of Amyloid-β Monomers and Oligomers: An Fcs, Sers and Afm Study**

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Alzheimer's Amyloid-β (Aβ) peptide changes its conformation as it aggregates (1) and acquires toxicity, ultimately causing Alzheimer's disease. Since it is difficult to decouple conformational changes from aggregation, any cause-effect relationship between them remains poorly explored. Here we attach Alzheimer's Amyloid-β40 monomers to silver nanoparticles, preventing their aggregation, and study their conformation under aggregation-favoring conditions using SERS. Surprisingly, the α-helical character of the peptide remains unchanged

between pH 10.5 and 5.5, while the solubility changes >100x. We infer that amyloid aggregation can start without significant conformational changes.
A significant part of the aggregation / organization of this extra cellular peptide probably takes place in the in the lipid membrane. The affinity of the monomers and oligomers for the lipid membrane, and the conformation that they adopt there, remain open questions. We have recently found that Aβ exhibits a strong enhancement in its membrane-affinity as it transforms from monomers to oligomers (2). Using Fluorescence Correlation Spectroscopy, we have now established a rapid and quantitative assay for this affinity. Further, using SERS of lipid bilayer coated gold nano-particles, we are currently investigating the conformation of Aβ in lipid environments.
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**1372-Pos Board B102**
**Transthyretin Interacts with Amyloid-β Oligomers to Delay Amyloid Aggregation**
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Protective effects of Transthyretin (TTR) ameliorate Alzheimer's pathology in mice models. This suggests a possible route for cellular defense mechanism against toxic effects of protein misfolding and aggregation. This view is further supported by the results from in vitro experiments showing direct interactions between TTR and amyloid-β (Aβ) peptides. However, the molecular mechanism of the TTR-Aβ interactions is currently unknown primarily due to heterogeneities associated with self-association of both TTR and Aβ. Here we investigate the mechanism of TTR-Aβ interactions by examining the effects of WT-human, a monomeric mutant and murine TTR on the kinetics of aggregation of both Aβ1-40 and Aβ1-42. The three of forms of TTR differ in terms of stability as tetramers or monomers, e.g., the WT-human and murine TTR are primarily tetramers but the tetramers of murine TTR are more stable. To monitor the aggregation of Aβ but not of TTR we use a recently developed aggregation assay based on the fluorescence quenching of tetramethyl rhodamine (TMR)-labeled Aβ. Our data indicate that all three TTR variants delay aggregation of both Aβ1-40 and Aβ1-42. However, the effects are strongest for the monomeric mutant and weakest for the murine TTR. Kinetic measurements of fluorescence resonance energy transfer between native tryptophan residues of TTR and the EDANS-labeled Aβ indicate that binding of TTR is dependent on the aggregation status of Aβ with maximal binding occurring to larger aggregates of Aβ. Additionally, the modulatory effects of TTR are stronger on Aβ1-42 than on Aβ1-40 consistent with the known higher oligomerization propensity for Aβ1-42. Taken together our results indicate that the tetramers of WT-human TTR dissociates to monomers to bind to the aggregation intermediates of Aβ to subsequently slow down the growth of these intermediates.

**1373-Pos Board B103**
**Amylin Interacts with Aβ and May Accelerate the Development of Dementia**
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Epidemiologically, type-2 diabetes (T2D) doubles the risk for dementia linked to cerebrovascular disease and/or Alzheimer's disease (AD). We have recently found that, in addition to plaques laden with Aβ, the brain of demented T2D patients also contains large deposits of amylin, a protein that makes up the pancreatic amyloid in T2D. Deposition of amylin (or islet amyloid polypeptide; IAPP) is promoted by hyperamylinemia, a key component of the metabolic syndrome. In the brain, amylin was identified in the blood vessel wall, perivascular space and tissue parenchyma. Moreover, we found amylin forming the core protein deposit of some amyloid plaques or co-localized with Aβ in combined plaques suggesting an amylin-Aβ pathology. Intriguingly, amylin deposition was also detected in brain specimens from patients with AD without clinically apparent diabetes. Amylin pathology in AD brain was similar to that in brain samples from the T2D patients group, including buildup on blood vessel walls and parenchyma. In contrast, brain specimens from age-matched healthy humans show only sporadic amylin deposits in blood vessels and brain parenchyma. Cerebral deposition of amylin in non-diabetic AD patients may be due to insulin resistance, which is common in aging.
To test the impact of hyperamylinemia on brain function, we use a rat model of T2D expressing human amylin in the pancreas (the HIP rat). We found that the infiltration of oligomerized amylin in cortical arteries induces lipid peroxidation and triggers an inflammatory response. As a result, HIP rats display changes in spontaneous activity and coordination. In contrast, rats matched for weight, glucose and age, but expressing only the *non-amyloidogenic* rat amylin, show no amylin accumulation and no behavioral changes.
In conclusion, hyperamylinemia promotes amylin deposition in the brain contributing to the development of cerebrovascular injury and neurological deficit.

**1374-Pos Board B104**
**Mapping the Structure of Tau Using Single Molecule FRET**
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Tau is a non-motor microtubule-associated protein which functions to maintain microtubule stability, primarily in the axons of neurons. The loss of this normal function, along with the aggregation and deposition of tau as neurofibrillary tangles, contributes to tau pathology in various neurodegenerative disorders, including Alzheimer's disease. Moreover, tau is reported to be an important regulator to motor proteins in microtubule-based transport. While interactions between tau and microtubules are of great interest, a detailed structural characterization is lacking. Tau is intrinsically disordered, lacking stable secondary and tertiary structure in solution, which makes it challenging to investigate its structural features by conventional techniques. Here we use single molecule Foster Resonance Energy Transfer (FRET) to probe the structure of tau bound to microtubules in order to establish a structural framework for the mechanism by which tau stabilizes microtubules. These measurements will not only provide insight into functional aspects of tau but also into the loss-of-function relevant to disease.

**1375-Pos Board B105**
**Exploring a Two-Step Adsorption of An Intrinsically Disordered Peptide at Model Templates**
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Biological processes offer several intriguing examples of directed self-assembly into higher order structures that influence human health and well-being. The aggregation of microtubule associating protein Tau, an intrinsically disordered soluble protein that forms neurofibrillar tangles in cells in Alzheimer's disease may be directed by the presence of hydrophobic templates. Using various lipid monolayers and lipid-free surfaces we monitor the adsorption and aggregation kinetics of Tau proteins to these model membranes. Monitoring the surface tension change shows a two-step adsorption process followed by these proteins. A novel microrheology and quartz crystal microbalance study shows that the first step is dominated by the N-terminus of peptide, while the second step is possibly due to an intermediate hair-pin structure. Our microrheology data also show that the viscoelastic properties of these proteins is dominated by the proteins propensity to aggregate.

**1376-Pos Board B106**
**Increased Affinity for Tubulin Impairs Tau Function**
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Tau is a microtubule binding protein that forms pathological aggregates in the brain in Alzheimer's disease and other tauopathies. Disease etiology is thought to arise from loss of native interactions between tau and microtubules, as well as from gain of toxicity tied to tau aggregation, although neither mechanism is well-understood. We have investigated the link between function and disease using disease-associated and designed disease-motivated mutants of tau. We used fluorescence correlation spectroscopy (FCS) to measure tau binding to free tubulin. We find that while the mutants bind stabilized microtubules with comparable affinities, they demonstrate an increased affinity for tubulin dimers. Morover, the mutant forms of tau are impaired in their ability to promote microtubule assembly. Using single molecule FRET, we measure conformational changes in tau upon binding to tubulin that provide a structural framework for the observed altered affinity and function. We propose a model that describes tau binding to tubulin dimers and a