Intrinsically Disordered Proteins (IDP) and Aggregates I

309-Pos  Board B89
Anomalous Stiffness Changes of Tau Protein in X-ray Single Molecule Observations
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Alzheimer Disease (AD) is one of the most famous neurodegenerative disorders, and the neuronal tangles serve as a hallmark of AD. They are composed of paired helical filaments of hyperphosphorylated Tau proteins, which are the microtubule-associated protein [1]. In order to understand the functions of a protein, we must determine its structures. But Tau protein’s structure hasn’t been identified because it is famous for being one of the intrinsically disordered proteins (IDP), which lack stable tertiary structures or secondary structures [2].

Here we observed Tau protein’s fluctuations with the Diffracted X-ray tracking (DXT). This method is able to monitor the tilting and twisting motions of single protein molecules with nanometer resolution. In this method, gold nanocrystals are attached to target protein molecules to probe their intramolecular motions, and these proteins are irradiated with synchrotron X-ray to get time displacements of Laue diffraction patterns from gold nanocrystal. As a synchrotron X-ray beamline, we used KEK NW-14A and SPring-8 BL40XU in Japan. In our experiment, in order to reveal the relation between an aggregation process and hyperphosphorylated Tau proteins, we phosphorylated wild-type and mutated recombinant Tau proteins with GSK-3β. Threonine and serine sites of these mutated Tau proteins were converted into Alanine in order not to be phosphorylated. And we found tau protein molecules were fluctuating between 0.3-1 nm in aqueous solution when a shutter speed was 36 ms/frame. More importantly, their fluctuations decreased after phosphorylated by GSK-3β. Finally, we specified the phosphorylation sites that affect structural fluctuations of Tau proteins.


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Simulation of the Distribution of Disordered Tau Protein Around Its Amyloid Fibrils Core
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Tau is an intrinsically disordered protein (IDP) implicated in Alzheimer’s disease. Tau protein has 240-aa-long N-terminal domain, 125 aa microtubules binding repeat domain, and 72 aa C-terminal domain. The tau fibril core is formed by the repeat domain, and both N- and C-terminal domains remain disordered in tau amyloid. There are experimental indications that these disorder segments form fuzzy coat that resembles a two-layered polyelectrolyte brush around tau fibril core. Previously, we have shown that tau fibrils formed by the repeat domain K18 protein have polymorphic amyloid state. In this work, using both all atom and coarse-grained Martini models, we simulated the full length tau amyloid structure to investigate the distribution of disordered tau proteins around its amyloid fibrils core. Our results provide insights into the organization of full length tau fibrils.

311-Pos  Board B91
Tau Filament Length Distribution Reflects End-To-End Annealing
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Tau is a microtubule associated protein that normally functions as a monomer in league with the microtubule cytoskeleton. However, in Alzheimer’s disease (AD), tau aggregates to form filamentous inclusions in cell bodies (neurofibrillary tangles, NFT) and cell processes (dystrophic neurites and neuritoid threads). The appearance of tau-bearing lesions correlates with neurodegenerative and cognitive decline, consistent with a connection between tau aggrega-
tion and disease progression. Indeed, in biological models, tau aggregates are toxic, with potency inversely proportional to aggregate size. However, the relationship between these species and the aggregation pathway is not well understood.

Here we investigate the aggregation mechanism of tau protein in vitro in an effort to identify interactions that manifest size dependence. The fits of a mathematical model describing simple nuclearation elongation polymerization to aggregation time-series, consistently overestimated filament growth rate while underestimating filament length distribution, indicating the presence of a secondary process in the pathway. On the basis of filament mixing and shearing experiments, we identified end-to-end annealing as a novel secondary interaction of nascent tau filaments. With the addition of an end-to-end annealing term to the mathematical model in equilibrium with filament fragmentation, we found improved fits for both time series and filament length distributions. In addition to quantifying the intrinsic rate constants for annealing and fragmentation, the model provided evidence for their dependence on filament length. The results indicate that filament ends are active, and that their propensity to engage in homotypic interactions is length dependent. We propose that heterotypic interactions at filament ends are candidate mediators of toxicity in biological models.

312-Pos  Board B92
Parameter Distribution Analysis of Tau Fragment K18 Fibrilization
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Neural amyloid deposits of microtubule-associated protein tau are implicated in a number of neurodegenerative disorders, notably Alzheimer’s disease (AD) and chronic traumatic encephalopathy (CTE). Fibrillation of tau, and of amyloid-forming proteins in general, appears to involve nucleation-dependent polymerization wherein small concentrations of “nuclei” form initially, followed by the rapid, highly favorable addition of further monomer to nuclei/fibril ends. Secondary nucleation, the formation of nuclei from fibrillar material, is a particularly important determinant of amyloid formation kinetics. When the reaction is monitored, this type of behavior results in highly cooperative, sigmoidal fibrillation curves. A number of small molecules derived from natural products have been shown to inhibit tau amyloid formation, but our understanding of their mechanistic effects is largely empirical.

A thorough investigation of the kinetic and structural effects of these compounds could aid in the rational design of more potent, specific inhibitors. Toward this end, we are utilizing a combination of fluorescence spectroscopy, mathematical modeling and numerical simulation to evaluate the heparin-induced fibrilization of a fragment of tau, K18. This strategy enables us to examine entire distributions of model parameter values that describe the data with comparable accuracy, as opposed to the conventional approach of identifying a single “best-fit” set of parameters. Both for experimental K18 fibrillation timecourses and for simulated sets of test data, the parameter distribution approach appears to better reflect the true experimental uncertainties involved in studies of amyloid formation than conventional least-squares fitting. These parameter distributions are sensitive to relatively small changes in the underlying kinetic rates, and we discuss how they can be used to assign detailed effects to known small molecule inhibitors of tau amyloid formation in an effort to generate more detailed models for their mechanisms of action.

313-Pos  Board B93
How Unfolded is Tau?
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Tau proteins regulate the dynamics, stability and transport properties of cytoskeletal scaffolding microtubules. Functional tau monomer has been classified as an intrinsically disordered protein. However, the degree of foldedness of the protein and intrinsic capability of the protein to gain rigidity is not well understood. We employed fluorescence, circular dichroism (CD), and Fourier transform infrared (FTIR) spectroscopic techniques to evaluate the foldedness of nascent tau filaments. With the addition of an end-to-end annealing term to the mathematical model in equilibrium with filament fragmentation, we found improved fits for both time series and filament length distributions. In addition to quantifying the intrinsic rate constants for annealing and fragmentation, the model provided evidence for their dependence on filament length. The results indicate that filament ends are active, and that their propensity to engage in homotypic interactions is length dependent. We propose that heterotypic interactions at filament ends are candidate mediators of toxicity in biological models.

Fluorescence data with bis-ANS reveals that in acidic buffer (pH 3.3), the protein contains predominantly a molten globular structure, while the rigidity degree partially collapse to a pre molten globular state in neutral and alkaline buffers. Exploiting the tyrosine fluorescence at similar solvent conditions suggests a three-dimensional structured domain(s) exists in soluble tau. Under moderate solvent changes, the soluble protein can adopt higher β-turn content, and extended/β-sheet structures compared to the dominant disordered structure of soluble tau in neutral buffer. Our CD data did not reveal the presence of extended helix polyproline II structure.

In conclusion, due to the flexibility and potential ability of soluble tau to gain or lose rigidity, microtubule-binding interactions that occur through specific folding is certainly a strong possibility. Furthermore, regardless of the
oligomerization behavior of tau, it is feasible that loss of foldedness and increased rigidity of the secondary structure content of soluble protein may contribute to a loss-of-function and eventually causes cytotoxicity, independent of intramolecular association.

314-Pos Board B94
Disease Related Point Mutations and Solution Conditions Determine Fibrillation Behavior of α-Synuclein
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In vitro fibrillation of proteins into amyloid fibrils provides critical insights into the factors influencing protein aggregation and has a key role in understanding the molecular basis of several neurodegenerative diseases caused by amyloids. α-Synuclein (αSyn), an intrinsically disordered protein implicated in Parkinson’s disease, aggregates readily in vitro into fibrils that exhibit appreciable structural polymorphism. This inherent polymorphism is a major obstacle in elucidating structural features and understanding the fibrillation process. By selecting specific solution conditions we were able to produce morphologically homogeneous fibrils of wt and disease mutant αSyn at the plateau phase of Thioflavin-T (ThT) assays, as evident from atomic force microscopy (AFM) imaging and analyses. Our results indicate that the in vitro aggregation conditions as well as the disease related point mutations of the protein determine the dominant morphology and the maturation behavior of the fibrils produced. Specifically, the morphology of wt αSyn fibrils appears to be dictated by two distinct mechanisms that is competitive growth of different polymorphs during the fibrillation phase followed by structural rearrangements during the process of aging. In contrast, the disease mutant αSyn variants aggregate with faster kinetics and result in fibrils with well defined and stable morphology over time. Additional cross seeding experiments of wt αSyn with disease mutant proteins have shown faithful transmission of the mutant fibril morphologies across two generations. The aggregation into homogeneous fibril populations with mutant-specific morphology is characterized by distinct fibrillation kinetics in ThT assays. Moreover, our experiments indicate differential interaction of ThT with morphologically different αSyn amyloid fibrils.

315-Pos Board B95
Fibril Breaking Accelerates α-Synuclein Fibrillation
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The formation of amyloid fibrils of α-synuclein is a pathological hallmark of the Parkinson’s disease. The fibrillation is an autocatalytic process that is seeded by mature αSyn fibrils. We studied dependence of the fibril growth rate on the concentrations of monomers and seeds and on mechanical shaking intensity and proposed a mechanism of α-synuclein aggregation that includes monomer binding to fibril ends and formation of new growing centers by fibril breaking. Such an autocatalytic fibrillation mechanism accounts for distinctive features of the experimentally observed fibrillation process. The autocatalytic growth of the fibril population at the beginning of (seeded) aggregation - the linear dependence of the observed aggregation rate constant on the square root of monomer concentration - strong acceleration of aggregation by shaking. Based on the experimental distribution of fibril lengths we expect that fibril breaking is random and that the probability of breaking is proportional to the fibril length. The relatively low efficiency of the formation of primary fibrils explains the highly stochastic nature of the observed lag time compared to the aggregation rate. The rate constant of monomer binding to fibril end could was calculated based on the aggregation rate and the average length of formed fibrils and corresponds to attachment of monomer to particular fibril end approximately every 10s. Aggregation rates at low concentrations show that binding of monomer to the fibril ends is a reversible process with equilibrium dissociation constant (Kd) less than 3 μM. The proposed model provides a quantitative means to compare α-synuclein aggregation rates and affinity to fibril ends under different conditions, and could be useful in characterizing and designing aggregation inhibitors.

316-Pos Board B96
Exploring the Phase Space of Alpha-Synuclein with Replica Exchange Simulations
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α-Synuclein (αSyn) is a intrinsically disordered protein involved in the pathogenesis of Parkinson’s disease, which is known to form toxic aggregates as well as fibrils. From a computational point of view, it represents a challenging system, as the protein can be found in multiple conformers, separated by many high free energy barriers. To overcome these barriers, we conducted replica exchange molecular dynamics. Using the Amber force field in implicit solvent for the αSyn monomer, we find transitions between states with both alpha helix, beta sheet and disordered structure. We also conducted molecular dynamics simulations for AS fragments to study the propensity for fibril formation. The results will be discussed in the context of existing and recent experimental findings.

317-Pos Board B97
Single Molecule Fluorescence Assay of Alpha Synuclein Dimerization
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The aggregation of α-Synuclein (α-Syn) is linked to Parkinson’s disease. The mechanism of early aggregation steps and the effect of pathogenic single point mutations remain elusive. We report here a single molecule fluorescence study of α-Syn dimerization and the effect of pathological single point mutations. Specific interactions between covalently immobilized fluorophore-free α-Syn monomers on a substrate and fluorophore-labeled monomers diffusing freely in solution were observed using total internal reflection fluorescence microscopy. The results showed that WT α-Syn dimers adopt two types of dimers, type 1 and type 2. The lifetimes of type 1 and type 2 dimers were determined to be 197 ± 3 ms and 3334 ± 145 ms, respectively. All three single point mutations, A30P, E46K and A53T, increased the lifetime of type 1 dimer as well as enhanced the relative contribution of type 2 dimer in the overall population with type 1 dimer being the major fraction. The kinetic stability of type 1 dimers (expressed in terms of lifetimes) had the following order A30P (693 ± 14 ms) > E46K (292 ± 5 ms) > A53T (226 ± 6 ms) > WT (197 ± 3 ms). More stable, type 2 dimers had lifetimes in the range of several seconds. The strongest effect, observed for the A30P mutant, resulted in a lifetime 3.5 times higher than WT for type 1 dimer. Moreover, the stability of WT dimers at pH 5 (938 ± 15 ms) was substantially enhanced compared with that at neutral pH. Our data suggests that α-Syn dimers are heterogeneous, and single-point mutations and acidic pH promote dimerization as evidenced by longer lifetimes. It also suggests that heterogeneity of the α-Syn dimerization could give rise to different aggregation pathways.

318-Pos Board B98
Alpha Synuclein’s Anomalous Structural Fluctuations in X-ray Single Molecule Observations
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The structures of alpha-synuclein (α-syn) in solutions resemble that of a random coil because α-syn is one of the intrinsically disordered protein (IDP). Alpha-syn is of great interest to Parkinson’s researchers because it is a major constituent of Lewy bodies. Here, we observed characteristic of structural fluctuation of wild type (WT), phosphorylated mimic (S129E) and familial mutants (A53T, E46K) by using Diffraction X-ray Tracking (DXT) as x-ray single molecule detection systems. DXT is a method of measuring internal motions of the proteins by using trajectories of labeled gold nanocrystal. In order to measure movements of the specific binding sites of proteins, DXT monitors of X-ray diffraction spots from gold nanocrystal that were labeled in the active sites of the proteins. We measured structural fluctuations of individual α-synuclein molecules with both high time-resolution (36ns/frame and 0.1ms/frame) and high precision (0.1nm scale). DXT experiments used the energy of quasi-white x-rays (energy peak-width of 2%, 10-20 keV, BL40XU, SPring-8). As a result, we discovered that WT’s fluctuation is lower than those of other mutants (S129E, A53T and E46K) from observed dynamical motion’s histograms. Furthermore, we found that modes of motions in E46K and A53T have closer to that of S129E than that of WT. From motion’s histograms in resonance direction for the chain axis of α-syn’s amino acid, we confirmed that motion’s histograms in WT has simple single Gaussian distribution. However, those in other mutants have complex ones. From our DXT results, it is very clear that there is specific different motions between WT of α-syn and other mutants. In addition to, we found that there is different number of X-ray diffraction spots from gold nanocrystal between WT of α-syn and other mutants.