identified the influence from the types of FG nups and inter-molecular spacing. FG nups showed a strong dependence of inter-molecular interaction strength on molecular spacing. However, inter-molecular interaction for one type is weaker compared to that of another type. While most of the FG nups of the two types are mainly disordered despite inter- and intra- molecular interactions, we observed noticeable beta-sheet formation at certain inter-molecular spacings, indicating a significant role between inter-molecular interaction and secondary structure formation.

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Aß Monomers Transiently Sample Oligomer and Fibril-Like Configurations: Ensemble Characterization using a Combined MD/NMR Approach

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Amyloid ß (Aß) peptides are a primary component of fibrils and oligomers implicated in the etiology of Alzheimer's disease. However, the intrinsic flexibility of these peptides has frustrated efforts to investigate the secondary and tertiary structure of Aß monomers, whose conformational landscapes directly contribute to the kinetics and thermodynamics of Aß aggregation. In this work, de novo replica exchange molecular dynamics (REMD) simulations on the μs/replica timescale are used to characterize the structural ensembles of Aß42, Aß40, and M35-oxidized Aß42, three physiologically prevalent isoforms with substantially different aggregation properties, the latter of which has hitherto not been investigated with computational techniques. Further, comparisons of J coupling data calculated from the REMD trajectories with their corresponding experimental values are used to validate these simulations and monitor equilibration. Our analysis indicates that all simulations converge on the 100 ns/replica timescale toward ensembles that yield good agreement with experimental J couplings. Here, we describe Aß monomers that are far more structured than other computational studies that rely on characterizations made on smaller timescales. Prominent in the C-terminus are antiparallel ß-hairpin topologies between L17-A21, A30-L36, and V39-A41, reminiscent of oligomer and fibril models, that expose the composite hydrophobic side chains to solvent and may serve as hotspots for self-association. A persistent V24-K28 bend motif is observed in all three species that is stabilized by buried backbone to side chain hydrogen bonds with D23 and a cross-region salt bridge between E22 and K28, highlighting the role of the side chain identities of the FAD-linked E22 and D23 residues in Aß monomer structure. These characterizations help illustrate the conformational landscapes of Aß monomers at atomic resolution and provide insight into the early stages of Aß aggregation pathways.

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The Functional Dependence of the Intrinsically Disordered Domains of Tau

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Hyperphosphorylated tau is the precursor to neurofibrillary tangles, one of the hallmark symptoms of Alzheimer's disease [1]. In its non-diseased state, the microtubule-associated protein tau promotes the assembly of tubulin into microtubules and regulates axonal transport. However, dysfunction of tau has been implicated in a class of diseases known as tauopathies, of which Alzheimer's is the most pervasive [2]. Characterization of tau has proven to be difficult, due to the presence of intrinsically disordered domains in the N- and C-termini of tau which precludes protein crystallography [3]. Using solution small-angle x-ray scattering, we examined tau-induced bundles of microtubules in cell-free conditions. By this method, we directly measured the inter-microtubule spacing by tau, the magnitude of the attractive force of tau, and the domains of the N- and C- termini that are responsible for the bundling of microtubules.

The six isoforms of tau are the result of alternative splicing and are commonly described as sequentially having an N-terminal (consisting of a projection domain and proline-rich region), microtubule-binding repeats, and a C-terminal. To examine the functional dependence of the projection domain, proline-rich region, and C-terminal, distinct tau constructs were expressed with deleted domains. Using the wild-type isoforms, the inter-microtubule spacing was found to be modulated by the length of the projection domain. Surprisingly, the use of the deletion constructs showed that neither the projection-domain or proline-rich region is necessary for bundling, in conflict with existing models [4].

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Determination of the Rate Constants of Oligomerization of Amyloid-β Peptides using Fluorescence Quenching of Tetramethylrhodamine

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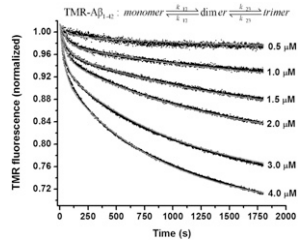
Soluble oligomers of amyloid-β (Aβ) play a central role in the pathology of Alzheimer's disease. Here we have determined the rate constants of the monomer-oligomer process of Aβ using a new method based on self-quenching of tetramethylrhodamine (TMR) which is covalently labeled at the N-terminal of Aβ. During aggregation TMR fluorescence is quenched in a time dependent manner in three distinct phases, an early oligomerization phase, a lag phase and a growth phase. Kinetic data of the early phase are consistent with a monomer-dimer-trimer process. The rate and the equilibrium constants differ markedly between $A\beta_{1-42}$ and $A\beta_{1-40}$ showing higher oligomerization propensity for A_{1-42}.The association rate constants ($\sim 10^2\ M^{-1}s^{-1}$) for both the peptides are slow compared to a diffusion limited process. The rates of oligomerization are altered dramatically with increase of temperature and are highly sensitive to changes in pH especially below pH 7.5. Our results for the first time provide quantitative estimation of the kinetics of monomer-oligomer process of Aβ. The methodologies presented here are simple and therefore can be easily applied to other amyloidogenic proteins.



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Inhibition and Mechanism of Islet Amyloid Polypeptide Toxicity

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A critical aspect of type II diabetes is the dysfunction and death of insulin-secreting β-cells. A 37-residue peptide hormone, islet amyloid polypeptide (IAPP), plays a central role in this pathology since it is known that rodent models transgenic for human IAPP show spontaneous development of β-cell dysfunction and diabetic symptoms. Recent evidence suggests that a subset of several alternative α-helical membrane-bound oligomers on-pathway to amyloid fiber formation, and not the amyloid fibrils themselves, are the toxic species in disease. We recently showed that IAPP adopts cell and membrane penetrating properties that are likely correlated with its toxicity towards β-cells; IAPP localizes to lysosomes of rat insulinoma pancreas β-cells (INS-1) at peptide concentrations determined by cell viability assays to be nontoxic, and relocates to mitochondria at toxic peptide concentrations (M. Magzoub and A. D. Miranker, *Faseb J.*, 2011, *26*, 1228-1238). Here, we evaluate the ability of small molecules to agonize and antagonize IAPP toxicity towards INS-1 cells via large-scale screens of cell viability. Localization of IAPP to subcellular compartments of INS-1 cells in the presence of these molecules is also investigated. We further describe the activity modulation and subcellular localization of the peptide in the presence of small molecules from libraries at Yale Small Molecule Discovery Center that have been shown to slow lipid-catalyzed amyloid fiber formation of IAPP.

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Screening and Design of New Modulators of IAPP Membrane-Binding and Toxicity

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Type 2 Diabetes Mellitus (T2DM) is characterized by pancreatic amyloid aggregates composed of islet amyloid polypeptide (IAPP; also called amylin), a small, intrinsically disordered peptide hormone. Pancreatic β-cell death due to membrane disruption mediated by non-amyloid or pre-amyloid oligomers of IAPP is thought to be central to the progression of T2DM. However, the rational design and screening of small-molecule and peptide ligands targeting these oligomeric states of IAPP is challenging using current pharmacological and biophysical methods. We recently used intermolecular single-pair Förster resonance energy transfer (spFRET) combined with constrained Monte Carlo