be confirmed experimentally. Using TIRF-microscopy and laser tweezers assays to measure microtubule self-assembly from GMPCPP-tubulin in vitro with nanoscale accuracy, we find that the off-rate is not constant but rather increases with increasing free subunit concentration. Consistent with this observation, we find that a simple two-dimensional (2D) model predicts the increasing off-rate with subunit concentration due to a shift in tip structure from relatively blunt at low concentrations to relatively tapered at high concentrations, which we confirmed experimentally by TIRF-microscopy. Because both the on-rate and off-rate increase with free tubulin concentration, the 2D model requires an association rate constant that is an order-of-magnitude larger than the 1D model. We tested this prediction by measuring the variability in assembly rate, and found that the on- and off-rates are consistent with the 2D model predictions and are an order-of-magnitude higher than predicted by the 1D model. In summary, we find that the kinetic rates of microtubule self-assembly have been severely underestimated in the literature, by at least an order-of-magnitude. Because net growth results from a small difference between large on-rates and off-rates, the net rate can be significantly altered by weak microtubule associated protein and therapeutic drug interactions with the microtubule.

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Tension Directly Stabilizes Reconstituted Kinetochore-Microtubule Attachments

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Kinetochores are macromolecular machines that couple chromosomes to dynamic microtubule tips during cell division, thereby generating force to segregate the chromosomes. Accurate segregation depends on selective stabilization of correct 'bi-oriented' kinetochore-microtubule attachments, which come under tension due to opposing forces exerted by microtubules. Tension is thought to stabilize these bi-oriented attachments indirectly, by suppressing the destabilizing activity of a kinase, Aurora B. However, a complete mechanistic understanding of the role of tension requires reconstitution of kinetochore-microtubule attachments for biochemical and biophysical analyses *in vitro*. Here we show that native kinetochore particles retaining the majority of kinetochore proteins can be purified from budding yeast and used to reconstitute dynamic microtubule attachments. Individual kinetochore particles maintain load-bearing associations with assembling and disassembling ends of single microtubules for >30 min, providing a close match to the persistent coupling seen *in vivo* between budding yeast kinetochores and single microtubules. Moreover, tension increases the lifetimes of the reconstituted attachments directly, via a catch bond-like mechanismthat does not require Aurora B. Based on these findings, we propose that tension selectively stabilizes proper kinetochore-microtubule attachments *in vivo* through a combination of direct mechanical stabilization and tension-dependent phosphoregulation.

Platform BG: Protein Aggregates

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Alzheimer Aβ Amyloid Annular Fibrils: Insight Into Polymorphism

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Elucidating the structure of $A\beta_{40}/A\beta_{42}$ fibrils is of interest in Alzheimer's disease research because it is required for designing therapeutics that target fibril formation at an early stage of the disease. Based on the cryoEM measurements and on ssNMR data, we probe various amyloid fibril organizations. Our study demonstrates that the two-fold symmetry tubular $A\beta_{42}$ fibril model with the C-termini facing the external surface of the fibril has a cavity, while model with the C-termini facing the internal surface of the fibril shrinks the tubular cavity. Experimentally, data for the three-fold symmetry structure of the $A\beta_{9\text{-}40}$ fibril suggest formation of tight hydrophobic core through M35 interactions across the fibril axis and strong I31-V39 interactions between different cross-β units. Herein, based on ssNMR data, we probe various models with three-fold symmetry of the full-length $A\beta_{40}$. We revealed that the unique $A\beta_{40}$ triangular structure has a large cavity along the fibril axis. Finally, the N-termini in $A\beta_{40}/A\beta_{42}$ fibrils can assist in the stabilization of the fibrilby interacting with the U-turn domains or with the C-termini domains. Our findings point to the relevance of the cavity in oligomers which should be considered, when targeting oligomer toxicity. This project has been funded in whole or in part with Federal funds from the NCI, NIH, under contract number HHSN261200800001E.

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A solid-State NMR Study of Abeta Protofibrils

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Aβ(1-40) is the major fibril-forming peptide from Alzheimer's disease, which adopts a highly ordered β-sheet conformation upon aggregation into amyloid fibrils. Several techniques have provided a wealth of structural information on mature Aβ(1-40) fibrils. Yet, the complex formation process of mature fibrils is, from a structural point of view, not well understood. Here we use ssNMR spectroscopy to elucidate the structure of Aβ protofibrils. This analysis is possible since the binding of the antibody B10AP prevents the conversion of these metastable intermediates into mature fibrils. With a set of eight peptides with varying isotope labeling schemes, 30 residues distributed over the entire peptide sequence were covered. ^{13}C CPMAS spectra and 2D correlation experiments were recorded for unambiguous assignment of all carbons. From the conformation dependent chemical shifts we could identify peptide segments of stable secondary structure and evaluated the backbone structure using TALOS. Based on this data, Aβ protofibrils encompass residues 16-22 and 30-36 in β-sheet conformation. Further, three structural regions of the protofibrils present random coil-like chemical shifts, one (residues 23-26) as intermediate segment between the β-strands and two others at the peptide N- and C- terminus. Obviously the structural elements of mature Aβ(1-40) fibrils are already present in protofibrils, but the β-sheet regions apparently elongate during the fibrils conversion. Further information about the dynamics of these regions is provided by measurement of the strength of dipolar couplings, which are converted into an order parameter. Protofibrils show high order parameters (>0.8) within the β-strand regions, while the measured *S* values are below 0.8 at the termini. We never observed *S* values below 0.4 that would have indicated very high mobility. Thus, significant structural order exists also within those sequence segments that have chemical shift values corresponding to a random coil.

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Kinetics of Amyloid-Beta Monomer to Oligomer Exchange by NMR Relaxation

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Non-fibrillar oligomers of the amyloid-beta peptide may be the primary toxic species in Alzheimer's disease but detailed structural and kinetic characterization of these states has been difficult. Using NMR relaxation measurements, we observe the kinetics of exchange between monomeric and large, polymorphic oligomeric species of amyloid-beta. 15N and 1HN R2 data at multiple magnetic fields were recorded for several peptide concentrations subsequent to the establishment of a stable pseudo-equilibrium between monomeric and NMR-invisible soluble oligomeric species. The increase in 15N and 1HN R2 rates as a function of protein concentration is independent of nucleus and magnetic field and shows only a small degree of variation along the peptide chain. This phenomenon is due to broadening arising from the conversion of monomer to the NMR-invisible oligomeric species (''dark'' state). At a total amyloid-beta concentration of 300 micromolar, the apparent first-order rate constant for this process is 3 ŝ-1. Fitting the McConnell equations for two dipolar-coupled spins in two-site exchange to transfer-of-saturation profiles at two radiofrequency field strengths gives an estimate for koff of 73 ŝ-1 and transiently bound monomer 1HN R2 rates of up to 42 000 ŝ-1 in the tightly bound central hydrophobic region and ~300 ŝ-1 in the disordered regions, such as the first nine residues. The fraction of peptide within the ''dark'' oligomeric state undergoing exchange with free monomer is calculated to be ~3%.

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Investigating Protein Aggregation using Segmental Isotope Labeling and 2D IR Spectroscopy

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Protein misfolding and aggregation are important processes in numerous human diseases, but the detailed structural changes that occur therein evade traditional crystallographic and NMR-based techniques. Rapid-scan 2D IR spectroscopy, combined with heavy isotope labeling, provides information on local secondary structure and coupling between IR chromophores, which can be used to study systems that aggregate *in vitro* on a timescale of