single molecule fluorescence confocal microscopy and FRET, allowing the characterisation of oligomers present during aggregation of monomers and disaggregation of fibrils. Additionally, we have extended our single-molecule studies to examine the species formed during the co-aggregation of $A\beta_{1-40}$ and $A\beta_{1-42}$ to understand the interaction at physiological concentrations and ratios. The thorough detection and characterisation of these potentially toxic oligomeric species provides a basis with which to screen therapeutic agents and other modulators of aggregation *in vitro* which could inform *in vivo* studies in the future.

1. F. Chiti, C.M. Dobson, *Annu. Rev. Biochem.* 75, 333-366 (**2006**)
2. A. Orte *et al*, *PNAS*, 105, 14424-14429 (**2008**)

**1844-Plat**
**The pH-Dependence of Amylin Fibrillization**
**Andrei T. Alexandrescu**, Suman Jha, Jessica M. Snell, Sarah R. Sheftic.
University of Connecticut, Storrs, CT, USA.
In type 2 diabetics, the hormone amylin misfolds into amyloid plaques implicated in the destruction of the pancreatic β-cells that make insulin and amylin. The pH-dependence of fibril formation affects fibrillization kinetics, fibril morphology, and possibly toxicity. Amylin is stored in β-cell secretory granules at pH 5.5 and is released into the extracellular matrix at pH 7.4. There are two ionizable residues in amylin: the α-amino group and His 18. Our approach to measuring the $pK_a$ values for these sites has been to look at the pH dependence of fibrillization in amylin variants that have only one of the groups: NAc-amylin (for His18) and H18R (for the N-terminus). The fibril-specific dye thioflavin-T (ThT) was used to monitor kinetics. The $pK_a$ of His18 is lowered to 5.1 in the fibrils, compared to the random coil value of 6.5. The lowered $pK_a$ is due to the unfavorable burial of a charge, and electrostatic repulsion between positively charged His18 residues on neighboring amylin molecules in the fibril. For H18R, which can only be protonated at the N-terminus, we see a $pK_a$ ~8 due to the N-terminus but a second $pK_a$ of ~4.5 due to protonation of ThT. We confirmed this with the amylin-derived peptide NAc-SNNFGAILSS-$NH_2$ which has no titratable groups but still shows a $pK_a$ of 4.5 due to ThT. A $pK_a$ of 1.9 for free ThT was determined by NMR but with fluorescence we are monitoring the excited-state of the amyloid-bound dye which has a perturbed $pK_a$. By using alternative methods to follow fibrillization such as the dye Nile Red or turbidimetry we were able to distinguish between the effects. Large differences in reaction kinetics were observed between the different methods, due to charges on the dyes, which affect fibril formation much like charges on the protein.

**1845-Plat**
**The Deltak280 Mutation in the Tau Protein may Induce the Formation of Stable Abeta-Tau Aggregates in FTDP-17**
**Yoav Raz**[1], Juliane Adler[2], Daniel Huster[2], Tilmann Haupl[2], Bernd Abel[2], Yifat Miller[1].
[1]Ben Gurion University in the Negev, Beer Sheva, Israel, [2]Institute of Medical and Biophysics, University of Leipzig, Leipzig, Germany.
One of the main pathological symptoms for Frontotemportal Dementia and Parkinsonism linked to chromosome 17 (FTDP-17) is the accumulation of neurofibrillary tangles in the brain via amyloid aggregation of the Tau protein. It is well known that this process is accelerated in the presence of Aβ. However, the molecular mechanisms underlying the interactions between Aβ and Tau protein are currently not well understood. One of the most well-known mutations in the tau protein that is related to FTDP-17 is the ΔK280 mutant. In our work, we have studied the effect of this mutation on the tau protein's propensity for aggregation in the absence and with the presence of Aβ. We constructed a set of molecular oligomer models based on previous solid-state NMR measurements and molecular dynamics simulations. Our results suggest that with the presence of Aβ, the mutated tau - Aβ complex occupies a rugged conformational landscape. The mutated tau oligomers that are stabilized by Aβ oligomers, preferred to interact as double layer structures instead of single layer structures. However, in the absence of Aβ, the conformational landscape of mutated tau is characterized only by one preferred structure. These new insights cast light on the interactions between amyloids in FTDP-17(as well as in other neurodegenerative diseases) and may offer new strategies for future pharmacological studies that aim to ameliorate the interactions between amyloids in FTDP-17. The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2011) under grant agreement 303741.

**1846-Plat**
**Binding of FDDNP Biomarker to Alzheimer's Disease Aβ Peptide**
**Christopher Lockhart**, Dmitri K. Klimov.
George Mason University, Manassas, VA, USA.
Molecular biomarkers such as FDDNP can provide early diagnosis of Alzheimer's disease (AD). These biomarker ligands are designed to interact with aggregation-prone Aβ peptides implicated in AD onset. While numerous experimental observations have demonstrated FDDNP binding to Aβ *in vitro* and *in vivo*, molecular details of binding mechanism are currently unavailable. In our study, we use explicit solvent replica exchange molecular dynamics to examine FDDNP binding to $A\beta_{10-40}$ monomer in all-atom detail. We found that at both high and low concentrations, FDDNP binds with high affinity to Aβ sequence locations near the central hydrophobic cluster and the C-terminal. Analysis of ligand-Aβ interactions at both concentrations identifies hydrophobic effect as a main binding factor. However, with the increase in ligand concentration the interactions between FDDNP molecules also become important due to strong FDDNP self-aggregation propensity and few specific binding locations. As a result, FDDNP ligands partially penetrate the core of Aβ monomer forming large self-aggregated clusters. Ligand self-aggregation does not affect hydrophobic interactions as a main binding factor or the location of binding sites in Aβ. Lastly, using Aβ conformational ensemble in ligand-free water as a reference we show that FDDNP induces minor changes in Aβ secondary structure. However, FDDNP significantly alters the tertiary fold in a concentration dependent manner by redistributing long-range side chain interactions. Because FDDNP does not change Aβ secondary structure, we argue that it is likely to provide unbiased imaging of Aβ aggregates.

**1847-Plat**
**The Local Mechanical Properties of Lipid Bilayers are Altered by Amyloid-Forming Proteins**
**Justin Legleiter**, Kathleen A. Burke, Elizabeth A. Yates.
West Virginia University, Morgantown, WV, USA.
A vast number of protein misfolding diseases are characterized by the formation of nanoscale protein aggregates generally termed amyloids. Such diseases include Alzheimer's disease (AD), Huntington's disease (HD), and type 2 diabetes. While the precise mechanism by which amyloidogenic aggregates are toxic remains unclear, various amyloid-forming proteins interact strongly with lipid membranes. We investigated how mechanical properties of model total brain lipid extract bilayers are altered when exposed to different amyloid-forming proteins (Aß, huntingtin, synthetic polyQ peptide, and amylin) utilizing in situ tapping mode atomic force microscopy (AFM) and scanning probe acceleration microscopy (SPAM). The advantage of the SPAM technique is that provides nanoscale spatially resolved maps of tip/sample tapping forces, which are directly correlated to mechanical properties of the surface. As a result, mechanical changes of lipid membranes can be mapped and correlated with changes in surface topography associated with protein aggregation. using this technique, we demonstrate that lipid bilayer structure is disrupted by amyloid-forming proteins. Disrupted regions of the bilayer were associated with decreased compression modulus and reduced adhesion to the AFM probe. Both of these observed mechanical changes are consistent with a decrease in the packing efficiency of the lipids within the bilayer. The interpretation of the mechanical changes in the lipid bilayers as measured by the SPAM technique were validated via numerical simulations of the tip/surface force interaction under a variety of conditions. These changes in bilayer mechanical properties associated with exposure to amyloid forming proteins may represent a common mechanism leading to membrane dysfunction in protein misfolding diseases.

**1848-Plat**
**Real-Time Visualization of a Pore-Forming Toxin Assembling on a Model Membrane**
**Neval Yilmaz**[1], Taro Yamada[1], Takayuki Uchihashi[2], Toshio Ando[2], Toshihide Kobayashi[1].
[1]RIKEN, Wako, Japan, [2]Kanazawa University, Kanazawa, Japan.
The assembly of the sphingomyelin (SM)-binding pore-forming toxin (PFT), lysenin, on SM/cholesterol bilayer was examined by high-speed atomic force microscopy (HS-AFM). Previous studies suggest that lysenin oligomerizes after binding to SM and forms a honeycomb structure. The HS-AFM images of SM/ cholesterol bilayer preincubated with lysenin exhibited the honeycomb assembly of the lysenin oligomers. The time-lapse AFM images revealed that the honeycomb formation took place quickly. During honeycomb formation most of the oligomers underwent reorganization either by dissociating into monomers or by rapidly diffusing along the membrane in less than a second. In the period of reorganization, the mobile oligomers arranged into a static, well-ordered lattice. Once this static layer was formed, the lysenin molecules were firmly bound to the SM/cholesterol bilayer and the oligomers neither dissociated nor diffused. Our results revealed the dynamic nature of the oligomers of a lipid binding toxin during honeycomb formation.