minutes to hours. Recently, we have utilized single-position 1-^{13}C=^{18}O labels to develop a detailed kinetic model of the aggregation of synthetic hIAPP, a 37 AA peptide implicated in type II diabetes mellitus. Here we demonstrate a segmental, ^{13}C labeling approach to the study the much larger human gammaD-crystallin, a 173 AA eye lens protein that aggregates via an unknown mechanism during cataract formation. Using bacterial expression and native chemical ligation, we have generated a gammaD-crystallin variant in which the highly similar N- and C-terminal domains are uniformly enriched in ^{12}C and ^{13}C, respectively. This results two well-resolved diagonal peaks in the 2D IR spectrum of the natively folded protein, with a separation of ~40 cm^{-1}. Aggregation under acidic conditions results in spectral changes consistent with amyloid fiber formation in the C-terminal domain and unfolding of the N-terminal domain, with evidence of interaction between the two based on the presence of cross-peaks. The structural model we propose, currently resolved at the level of individual domains, is nonetheless the most detailed available for a gammaD-crystallin aggregate to date. We anticipate that the method described will be broadly applicable to difficult systems including many aggregating, natively unstructured, or membrane-associated proteins.

2892-Plat
Structural Variations in the Aggregation Pathways of Normal and Pathological Huntingtin-Like Peptides
Tatiana Perevozchikova[1,2], Christopher Stanley[3], Helen P. McWilliams-Koeppen[4], Valerie Berthelier[4].
[1]Genome Science and Technology, University of Tennessee, Knoxville, TN, USA, [2]Graduate School of Medicine, University of Tennessee Medical Center, Knoxville, TN, USA, [3]Neutron Scattering Science Division, Oak Ridge National Laboratory, Oak Ridge, TN, USA, [4]Graduate School of Medicine, University of Tennessee Medical Center, Knoxville, TN, Knoxville, TN, USA.
The current challenge in the amyloid field is to obtain structural information on the progression of associated aggregation pathways, relate them to the cause of disease, and specifically point to the species truly responsible for neurotoxicity. Huntington's disease (HD) is linked to the aggregation of mutant huntingtin (htt) peptide, which contains an abnormally long polyglutamine (polyGln) repeat that leads to the formation of fibrils with stable, β-sheet rich structures. Analogous to other neurodegenerative diseases like Alzheimer's, mature htt fibrils are thought to be overall protective while the earlier smaller intermediates are proposed to be toxic to neuronal cells. We performed time-resolved small-angle neutron scattering (SANS) experiments to monitor the structural kinetics for htt peptides having polyGln repeats in the normal (22 Glns) and pathological (42 Glns) range. In addition to observing the expected faster aggregation rate for longer polyGln repeats, we find distinct conformational differences between normal and pathological htt. We also present, for the first time, three-dimensional structures of intermediates formed at the earliest stages of htt aggregation as detected by SANS and obtained using *ab initio* shape reconstruction methods. Finally, we are able to investigate the internal structure of the mature fibrils, where the mass-per-length of pathological htt fibrils is considerably less than normal htt fibrils. These findings provide the first steps toward characterizing the oligomers formed by htt peptides and illustrate the utility of SANS for identifying different aggregate intermediates formed in the development of neurodegenerative diseases.

2893-Plat
Time-Resolved Small-Angle X-Ray Scattering Study of the Early Formation of Amyloid Protofibrils on a Apomyoglobin Mutant
Maria Grazia Ortore[1], Francesco Spinozzi[1], Silvia Vilasi[2], Ivana Sirangelo[2], Gaetano Irace[2], Anuji Shukla[3], Theyencheri Narayanan[3], Raffaele Sinibaldi[1], Paolo Mariani[1].
[1]Università Politecnica delle Marche, Ancona, Italy, [2]Seconda Università di Napoli, Napoli, Italy, [3]ESRF, Grenoble, France.
Early prefibrillar intermediates are a key issue in amyloid researches, as they show the highest cytotoxicity with respect to mature fibrils, which are less toxic or even harmless. However, the early aggregation process is largely unknown, since nucleation events are rare, and elongation and reorganization processes are very fast. Time-resolved synchrotron Small Angle X-ray Scattering (SAXS), coupled to extended model fitting analysis, has been used to provide in a millisecond temporal range the structural and aggregational properties of the early and soluble protofibrils formed by a model protein after a pH jump. The amyloidogenic apomyoglobin mutant W7FW14F has been investigated as fibril former: this mutant, which is in a monomeric, partly folded state at acidic pH, undergoes at neutral pH a nucleation-dependent polymerization reaction, resulting in the formation of cytotoxic amyloid fibrils similar to those detected for proteins involved in amyloid diseases. SAXS evidenced that oligomerization of W7FW14F in solution happens in less than 100 ms after the pH jump from 4.0 to 7.0., while the resulting pattern of protein prefibrillation reveals the simultaneous presence of worm-like species and of cylindrically-shaped aggregates, whose structural features mainly change after 20 ms from the solution pH jump. Model fitting analysis gives the composition of the different oligomers and their relative concentration as a function of time, suggesting that protofibril formation occurs through the presence of aggregation and rearrangement competing processes and through the contribution of multiple coexisting elongated and worm-like protein species. The possibility to use SAXS in monitoring the effects of cosolvents and/or pharmaceutical agents in modifying or preventing the early amyloid aggregation patterns is then demonstrated.

2894-Plat
AFM Imaging and Single-Molecule Force Spectroscopy of Transthyretin Amyloid Intermediates
Ricardo H. Pires[1,2], Maria J. Saraiva[2], Ana M. Damas[2], Miklós S.Z. Kellermayer[1].
[1]Dept. of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary, [2]Institute for Molecular and Cell Biology, Porto, Portugal.
Aggregation of Transthyretin (TTR) in the form of amyloid fibrils is associated with systemic and neurological disorders. As part of the amyloid fibrilogenesis process, several oligomeric and protofibrillar intermediate structures have been identified and found to possess greater cytotoxicity than the mature amyloid fibrils. The structural dynamics and the molecular mechanisms behind the cytotoxicity of these intermediates are largely unknown.
In the present work we explored the structure, dynamics and mechanics of TTR amyloid intermediates with a combination of AFM imaging and single-molecule force spectroscopy. We show that annular oligomers display a tendency of association into a linear array. AFM imaging and force measurements indicated that annular oligomers are constituted by 8 TTR dimers that are largely unfolded when compared with native TTR. Associated annular oligomers undergo a collapsing structural transition before the appearance of amyloid protofibrils. The emerging TTR protofibrils display morphological features predicted by the double-helical model of the amyloid protofilament. Upon evoking their disassembly, protofibrils dissociated into annular oligomers. Thus, annual oligomeric species represent an important structural intermediate along both the assembly and disassembly pathways.
In this study we have dissected several structural transitions associated with the formation of TTR amyloid protofibrils. Because amyloidogenic aggregation is a potentially universal feature of proteins, the transitions reported here might be also relevant for other amyloidogenic disorders as well.

2895-Plat
Characterization and Single Molecule Conformational Studies of Soluble Alpha-Synuclein Oligomers
Adam Trexler, Elizabeth Rhoades.
Yale University, New Haven, CT, USA.
Alpha-Synuclein (αS) is a natively unstructured protein central to the pathology of Parkinson's disease. A growing body of evidence suggests that soluble oligomeric αS species are responsible for cell death which perpetuates Parkinson's. A variety of protocols have been described which populate oligomers *in vitro* for biophysical characterization, yet it remains unclear if these disparate protocols yield similar species and if these species are relevant to disease states. Furthermore, detailed structural analysis of these oligomers, which could be exceptionally useful towards elucidating mechanism of toxicity and avenues for disease treatment, remains difficult given their low abundance and apparent heterogeneity. In order to compare their bulk structural characteristics, we have prepared αS oligomers from a variety of protocols and characterized them using anti-oligomer antibody A11, cytotoxicity assays and colocalization studies with SH-SY5Y neuroblastoma cells, and ThT fluorescence to probe their ability to seed amyloid fibrillation. Our studies have identified a subset of oligomers which are on the order of 50 αS monomers in size, are A11-reactive, linking them to disease states *in vivo*, and are toxic to and readily taken up by cells in culture. We use single molecule Förster resonance energy transfer to measure the conformational states of individual αS monomers incorporated into oligomers. We have found in multiple regions of αS the protein apparently adopts a highly heterogeneous ensemble of conformations within oligomers, which emphasizes the suitability of single molecule techniques for their study. Through understanding the conformational states of αS before and after aggregation to toxic oligomeric species, we will provide insight into the mechanism of oligomer formation and toxicity.