possible beginning of nucleation. The study has provided a base for continuation of the study of oligomerization and subsequent fibrillation of BLG, which may provide a fundamental mechanism of formation transferable to other proteins in vivo.

1327-Pos
Amyloid Gels: Formation of an Insulin Fibrillar Network
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The formation of insulin amyloid fibrils is important not only for the development of reliable drugs and drug delivery systems, but also for modeling the basic properties of protein self-assembly. Fibrillation kinetics are typically characterized by an initial apparent lag-phase, related to the formation of oligomer, protofibrils and aggregation nuclei. Afterwards, aggregation proceeds over a wide range of length scales via fibril elongation, thickening and/or flocculation, and eventual gelation. Here, we focus on the formation of such a gel, made of insulin amyloid fibrils, upon incubation at high temperature and low pH. By light scattering and rheological techniques, we monitor the development of the structural, dynamical and mechanical properties of fibrillar aggregates, up to the dynamic arrest of the sample and the appearance of a non-ergodic behavior, which marks the occurrence of gelation. Atomic force microscopy imaging on incubated samples highlight the existence of a fibrillar network , as well as a complex hierarchy of different morphologies. Also, small and large angle dynamic light scattering experiments clearly show a non-diffusional dynamic behavior. Our experiments were able to reveal the structural details hidden in the apparent lag-phase, displaying the slow fibril nucleation and elongation. We confirm that this initial stage is followed by an exponential growth of structures of different sizes. These two kinetic stages of structural growth are mirrored by the kinetics of the viscoelastic properties and, in particular, by the growth of the elastic modulus. Our results show that the appearance of a noteworthy elastic network is associated with the initial fibril nucleation and elongation rather than with the formation of larger structures which cause the eventual gelation.

1328-Pos
Hybrid Amyloid Fibril Genesis with Components of Sporadic and Familial Alzheimer's Disease
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Generally, Alzheimer's Disease (AD) develops spontaneously. Nevertheless, many families with an inheritable occurrence were identified. Of these, a considerable number shows mutations in the Alzheimer Precursor Protein (APP) gene concerning regions of the Aß1-40 peptide, as e. g. the ''Flemish'' A21G [APP A692G] or the ''Iowa'' mutant D23N[APP D694N]. All except two of today's known Aß1-40 mutations in familial AD were reported heterozygously dominant. Therefore, an interaction of wild type (WT) and mutated (MUT) Aß peptide is likely to occur in the brain of affected patients.
Here we report on the extended co-fibrillation analysis of WT and MUT Aß1-40 peptide. Thioflavin-T fluorescence data of cofibrillation kinetics indicated a collateral aggregation process, correlated with the MUT:WT ratio. Coherently, the fibril morphologies of cofibrillates as observed in negative contrast transmission electron micrographs also appear to correlate with the MUT:WT peptide ratio.
We interpret these results that MUT peptide with a preferred fibril conformation can template the fibril formation of the morphologically pluripotent WT dose-dependently. This may explain the strain-like symptomatic of different familial AD cases.

1329-Pos
Iapp Preamyloid Oligomers Accumulate in the Heart and Contribute to Cardiac Dysfunction in Type-2 Diabetes
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Islet amyloid polypeptide (IAPP), a hormone co-secreted with insulin by pancreatic β-cells, forms amyloids when overexpressed. IAPP amyloids accumulate in pancreatic islets and are a hallmark of type-2 diabetes mellitus (T2DM). Recently, IAPP amyloids were found in kidneys of T2DM patients, suggesting that IAPP can deposit in organs other than pancreas. Apparently, the toxic effects to cells are mediated by the preamyloid oligomers. Here, we investigated whether IAPP preamyloid oligomers are present in the heart in T2DM. Using immunochemistry, we found significantly increased levels of IAPP oligomers (by 18 ± 3%) in failing hearts from T2DM humans vs. non-diabetics and in hearts from T2DM rats transgenic for human IAPP (by 88 ± 15%) vs. T2DM rats expressing only rat non-amyloidogenic IAPP. Most likely, IAPP accumulation in the heart occurs in the pre-diabetic state, **when** IAPP is oversecreted in the blood. To investigate the acute effect of IAPP oligomers on cardiac function, we incubated isolated rat cardiac myocytes with exogenous IAPP (5 and 50 μM) for 1-2 h. 50 μM IAPP significantly increased Ca transient amplitude (by 73 ± 19%) in myocytes contracting at 2 Hz. For rapid screening and analysis of sites susceptible for IAPP accumulation we designed a noninvasive water proton NMR protocol. We deciphered the magnetic signal of water surrounding preamyloid oligomers. The variation of this signal was correlated with population distributions of oligomers and fibrils by using immunochemistry and electron microscopy. We found that embryonic amyloids generate hyper-intense magnetic signals, which are distinct from hypo-intense magnetic signals induced by amyloid plaques. These results suggest that IAPP preamyloid oligomers contribute to cardiac dysfunction in T2DM. Water proton NMR may prove a useful non-invasive method for detecting these molecular entities in the heart.

1330-Pos
Small Molecule Inhibitors of Islet Amyloid Polypeptide Fibrillogenesis and Cytotoxicity
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Protein fiber formation is associated with diseases ranging from Alzheimer's to type II diabetes. The fiber formation is a complex reaction, which includes a number of conformational and oligomeric intermediate states. In recent years, it has become clear that it is these states, and not the end product (i.e. fibers) of amyloid formation that are the toxic agents associated with disease. These insights indicate that small molecule screens must to be directed at assembly mechanism in order to maximize the prospects for success. Islet amyloid polypeptide (IAPP) is a 37 residue peptide hormone co-secreted with insulin by the ß-cells of the pancreas. In patients with type II diabetes, this protein aggregates as amyloid in a process that is correlated with ß-cell dysfunction and the loss of ß-cell mass. In in vitro studies, the addition of soluble IAPP has been shown to be toxic to many ß- and non-ß-cell lines. IAPP fibrillogenesis, as is the case for many other amylodiogenic proteins, can be catalyzed by lipid bilayers. Paradoxically, while amyloid fibers are ß-sheet rich, membrane-stabilized states are α-helical. We have identified a small molecule alpha helix mimetic, IS5, which inhibits bilayer catalysis of fibrillogenesis, and rescues IAPP-induced toxicity in cell culture. Importantly, IAPP:IS5 interactions localize to the putative α-helical region of IAPP, revealing that α-helical states are on pathway to fiber formation. Normally, IAPP is not amyloidogenic as its cosecreted partner, insulin, prevents self-assembly. Here, we show that IS5 inhibition is synergistic with insulin. IS5 therefore represents a new approach to amyloid inhibition as the target is an assembly intermediate that may additionally restore functional IAPP expression.

1331-Pos
Zinc Inhibits Human Islet Amyloid Polypeptide (IAPP) Amyloidogenesis
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Human Islet Amyloid Polypeptide (hIAPP) is a highly amyloidogenic protein found in islet cells of patients with type II diabetes. Because hIAPP is highly toxic to beta-cells under certain conditions, it has been proposed that hIAPP is linked to the loss of beta-cells and insulin secretion in type II diabetics. One of the interesting questions surrounding this peptide is how the toxic and aggregation prone hIAPP peptide can be maintained in a safe state at the high concentrations found in the secretory granule where is stored. We show here through a combination of NMR, ITC, CD, and Thioflavin T fluorescence that zinc, which is found at millimolar concentrations in the secretory granule, binds to hIAPP with a Kd of approximately 100 nM and inhibits hIAPP amyloid fibrillogenesis in the micromolar range. NMR spectroscopy shows that zinc interacts with hIAPP through coordination to His18 and a probable cation - pi stacking interaction with Phe15. ITC binding experiments with the rat variant of IAPP, which lacks His18, indicated an additional binding site with approximately 1 mM affinity. The lower affinity binding site was localized to Arg11 by NMR. The binding of zinc also alters the structure of hIAPP, rigidifying the N-terminal region of the protein in a near-helical conformation stabilizing the non-amyloid form of the peptide. The inhibition of the aggregated and toxic forms of hIAPP by zinc provides a possible mechanism between the recent discovery of linkage between deleterious mutations in the SLC30A8 zinc transporter, which transports zinc into the secretory granule, and type II diabetes.