V. (2011) *PLoS One* 6, e18759). Here, we present the physiochemical aspects of LFAO-monomer interactions and its implications to AD. We discovered that LFAOs are a replicating strain of oligomers that recruit Aβ42 monomers and quantitatively convert them into LFAO assemblies at the expense of fibrils, a mechanism similar to prion propagation. These results further support the hypothesis that low molecular weight oligomers can be generated via non-fibril formation pathways. This unique self-replicating property of LFAOs has opened doors towards mechanisms that may be of profound significance for Alzheimer disease pathology.

250-Pos Board B19
Structural Evolution of Oligomeric Vs. Oligomer-Free Amyloid Fibril Growth
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Deposition of disease-specific proteins or protein fragments as fibrillar aggregates is the molecular hallmark of both neuropathic and systemic human diseases, including Alzheimer's disease, type II diabetes or rheumatoid arthritis. While the cross-β sheet architecture of mature fibrils is firmly established, insights into the structural features of intermediates formed transiently fibril assembly has been hampered by their intrinsic metastability. We show here that correlated thioflavin T (ThT) fluorescence and light scattering measurements and infrared spectroscopy can be used to monitor the structural evolution of amyloid intermediates of lysozyme emerging along an oligomer-free *vs.* oligomeric assembly pathway. While ThT fluorescence responded to all generations of transient intermediates, it did so with very different efficacies for the two different assembly pathways. Correlating ThT responses against light scattering indicated that the observed differences in ThT responses results from intrinsic differences (binding affinity, quantum yield) of ThT interactions with intermediates and mature fibrils in different pathways. Infrared spectroscopy confirmed that both assembly pathways result in the formation of cross-beta sheet structures early on. At the same time, there were reproducible spectral differences across these two pathways. All these pathway specific features emerged upon formation of the earliest intermediates and persisted up to late-stage fibrils. We confirmed that neither the existence of these two pathways nor their specific structural features were affected by the hydrolysis of lysozyme that occurs at highly acidic growth conditions. These observations imply that intermediates along different assembly pathways are not just morphologically but also structurally distinct and that these pathway-specific structural motifs become established during the very earliest aggregation events.
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251-Pos Board B20
Structural Characterization of Insulin Fibril Surfaces using Tip Enhanced Raman Spectroscopy (TERS)
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Amyloid fibrils are protein aggregates that are strongly associated with a variety of highly pathogenic diseases, such as Alzheimer's disease, Creutzfeldt-Jakob disease, Parkinson disease, etc. Direct studies of fibril surfaces become crucial for the understanding of the toxicity and pathogenity of amyloid fibrils, first of all, due to their direct contact with the aqueous media. Tip-enhanced Raman spectroscopy (TERS) is one of very few techniques, which allows to probe the fibril surface directly and with high spatial resolution.1 Our current findings indicate that surface of insulin fibrils is highly heterogeneous.2 More than 30% of the fibril surface is dominated by β-sheet, while the rest of it is composed of α-helix and unordered protein secondary structures. The propensity of various amino acids on the fibril surface and specific surface secondary structure elements were evaluated. β-sheet areas are rich in cysteine and aromatic amino acids, such as phenylalanine and tyrosine, whereas proline was found only in α-helical and unordered protein clusters. In addition, we showed that carboxyl, amino and imino groups are nearly equally distributed over β-sheet and α-helix/unordered regions. Overall, this study provides valuable new information about the structure and composition of the insulin fibril surface and demonstrates the power of TERS for fibril characterization.
(1) Deckert-Gaudig, T.; Kämmer, E.; Deckert, V. J. Biophotonics 2012, 5, 215-219.
(2) Kurouski, D.; Deckert-Gaudig, T.; Deckert, V.; Lednev, I. K. J. Am. Chem. Soc. 2012, 134, 13323-13329.

252-Pos Board B21
Sequence and Structural Modulators of Elastin Assembly and Mechanical Properties
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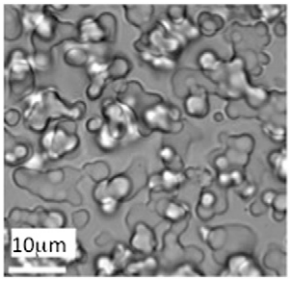
Elastin is the self-assembling extracellular matrix protein that provides elasticity to tissues. A fundamental requirement for forming elastomeric materials is retention of a high degree of conformational disorder even when aggregated. Elastin disorder is strongly related to a high (50%) combined proportion of proline and glycine residues within hydrophobic domains. The majority of elastin hydrophobic domains have an average proline spacing of 4-8 residues. However, the native sequence of hydrophobic domain 30 (D30) is uncharacteristically proline-poor. Here we investigated the contribution of D30 and variants to the self-assembly and material properties of elastin-mimetic biomaterials. Addition of native D30 substantially stabilized the surface of assembled aggregates compared to proline-rich controls, suggesting the potential for interfacial order in mediating droplet growth, rigidity and interactions. Materials were stiffer, consistent with a greater number of contacts between monomers, were less resilient, and displayed less internal resistance to force. Conversely, mechanical properties were restored upon addition of the longer and glycine-rich rat D30, suggesting an important contribution to conformational entropy from domain length and high glycine content. Taken together, we hypothesize structured motifs and cross-linking density play key roles in modulating elastin assembly and mechanics.



253-Pos Board B22
Anisotropic Models of Charged Protein Solutions
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We are currenctly developing a hybrid theoretical and numerical model for aggregate statistics of protein solutions as a function of ion density and pH. Our goal is to create a coarse-grained description, a qualitative model that captures the fundamental properties for a wide class of systems. We concentrate on modeling the field of inter-protein electrostatic interactions. There are numerous ways of doing this, all involving different levels of discretization and approximation to the fields and forces involved.
We use a non-linear Poisson-Boltzmann equation to fit a (small) set of discretized ''macro-charges'', a new fit for each solvent and pH. The idea is that these ''toys'' will give a rough idea of the thermodynamic quantities of a protein solution in bulk, pointing the way for more detailed experiments both in vivo and in silico. Many typical treatments for a large number of particles consider either an isotropic contribution or an all atom-approach (with fewer particles). We seek the median of these extremes to capture a bit more realism without expense of the models simplicity. Once the field has been approximated we run a battery of Monte-Carlo experiments to determine thermodynamic variables (such as the average energy, specific heat and the second virial coefficent). Qualitative structural parameters, such the radial distribution function are calculated and correlated to small angle scattering experiments.

254-Pos Board B23
Non-Native Structural Properties of the Glaucoma-Associated Olfactomedin Domain of Myocilin Lead to Amyloid Fibrils
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Approximately 4% of adult-onset and 10-33% of juvenile-onset primary open angle glaucoma cases are caused by mutations in the gene encoding for myocilin. The vast majority of mutations are within the 30 kDa C-terminal olfactomedin (OLF) domain. Disease-causing myocilin variants aggregate within human trabecular meshwork (HTM) cells instead of secretion to the extracellular matrix. This accumulation taxes the cells and leads to HTM cell death, resulting in increased intraocular pressure, and a hastening of glaucoma-associated vision loss. In spite of reports on the intracellular accumulation of myocilin in HTM cells and model organisms, these aggregates have not been structurally characterized. Our lab recently provided biophysical evidence for the hallmarks of amyloid fibrils in aggregated forms of WT and mutant myocilin localized to the C-terminal OLF domain (myoc-OLF). Under mildly

destabilizing conditions, WT myoc-OLF has a distinct non-native structure capable of forming amyloid fibrils. In the case of disease-causing myocilin variants, this non-native structure is accessible under physiological conditions, leading to accelerated fibril growth. Building on the hypothesis that partial unfolding of myoc-OLF is necessary for fibril growth, this study also investigates the structural and biophysical effects of adding stabilizing agents, such as osmolytes and Ca^{2+}, to the myoc-OLF domain as a strategy to prevent myoc-OLF amyloid aggregation. The results of this study suggest a novel protein-based hypothesis for glaucoma pathogenesis and the need for further testing in a clinical setting.

255-Pos Board B24

Understanding the Mechanism of Somatostatin-14 Amyloid Formation In Vitro

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Somatostatin-14 (SST-14) is a small cyclic peptide hormone secreted by hypothalamus, one of the well-known functions of which in the body is growth hormone inhibition. SST-14 has previously been reported to spontaneously self-assemble in water containing 150 mM NaCl into amyloid-like nanofibrils. Recently, it was shown that SST-14 forms amyloid in presence of glycosaminoglycan heparin at secretory granule relevant conditions. However, the pathway of its amyloid formation in not understood yet. In the present work, we studied the mechanism of SST-14 amyloid formation *in vitro* as well as *in silico*, in the presence and absence of heparin. Our *in vitro* data suggests that SST-14 in presence of heparin, transforms from its monomeric 'random coil' conformation to a secondary structure representing amyloid, via a 'helix-rich intermediate. It was observed in this study that SST-14 adopts a 'nucleation-dependent polymerization' mechanism of amyloid formation. We also studied the disulphide bond reduced 'non-cyclic' SST-14 (ncSST) form for amyloid formation, and observed that ncSST formed amyloid fibrils instantaneously in presence of heparin compared to the cyclic form. Furthermore, the ncSST fibrils were observed to possess higher conformational and thermal stability relative to the cyclic SST-14 fibrils.

Using all-atom molecular dynamics simulations, we studied molecular interactions among SST-14 peptides in the presence and absence of heparin. Our *in silico* results suggest that heparin induces aggregation of SST-14 by increasing local concentration of the peptides and promotes interpeptide contacts and secondary structure transformation in the peptides. Overall, this study offers the mechanism of somatostatin-14 oligomerization and amyloid formation, which has substantial value in understanding its storage within secretory granules.

256-Pos Board B25

Control of Amyloid Fibril Assembly by Cosolutes

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It is widely recognized that peptide assembly into fibrils (or amyloids) sensitively responds to conditions set by myriad cellular solutes. These cosolutes include two important classes: macromolecular crowders and compatible osmolytes. In effort to resolve the effect by which these cosolutes control peptide self-assembly, we have recently found that addition of macromolecular PEG only slightly affects fibril formation of a model peptide in vitro. Polyol osmolytes, in contrast, lengthen the lag time for aggregation, and lead to larger fibril mass at equilibrium. Model calculations suggest that all cosolutes similarly lengthen the time required for nucleation, possibly due to their excluded volume effect. However, PEGs may in addition promote fibril fragmentation, leading to lag times that are overall almost unvaried. Moreover, polyols effectively slow the monomer-fibril detachment rates, thereby favoring additional fibril formation. Preliminary results from coarse-grained molecular dynamics allow us to follow the first steps of protein assembly and nucleation. Input from experimental solvation thermodynamics of a model peptide are used to calibrate our simulations, and to test the effect of cosolutes on peptide assembly into proto-fibrils. Our analysis provides first hints that cosolutes act not only by changing association or dissociation rates of peptide self assembly, but potentially also by directing the formation of fibrils of varied morphologies with different mechanical properties. Importantly, hydration interactions are suggested as an integral component that directs this process. Although additional experiments are needed to unambiguously resolve the action of excluded cosolutes on amyloid formation, it is becoming clear that these compounds are important to consider in the search for ways to modulate fibril formation.

1. S. Sukenik, R. Politi, L. Ziserman, D. Danino, A. Friedler, and D. Harries, PloS one, 2011, 6, e15608.
2. S. Sukenik and D. Harries, Prion, 2012, 6, 26-31.

257-Pos Board B26

A Class of Ultrasmall Aliphatic Peptides Mimics Natural Fibrous Scaffolds by Self-Assembly

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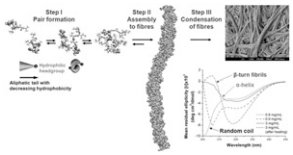
A class of ultrasmall tri to hepta peptides self-assemble into hydrogels, due to their characteristic sequence motif - an aliphatic amino acid tail of decreasing hydrophobicity capped by a polar head. These peptides organize themselves to supramolecular scaffolds of long helical fibers that extend to the µm-range. During self-assembly, these peptides form amyloid aggregates via unexpected α-helical intermediates, with the trimer as the shortest ever reported sequence with α-helical propensities. Circular dichroism and X-ray diffraction confirmed conformational changes terminating in thermo-stable β-type structures. Molecular dynamics simulations of peptide behavior in water revealed monomer antiparallel pairing and stably condensed coiled fibers. The assembled macrostructures are biocompatible and non-toxic. Their high mechanical strength can be tuned by adjusting salt and pH, paving the way for applications from injectable therapies to repair and replacement of damaged tissues. In particular, their biocompatibility towards a variety of human and other mammalian primary cells, qualifies them for future applications in tissue engineering and medical regenerative therapy. The ultrasmall size and the dynamic facile assembly process make this peptide class an excellent model system for studying the mechanism of amyloidogenesis, its evolution and pathogenicity.



258-Pos Board B27

Geometry and Mechanism of Fiber Formation: A Study of Peptide Segments of Amyloid Proteins

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Many soluble proteins self-assemble into fibrillar, β-sheet rich, stable aggregates that are each associated with a particular disease. It is thought that peptide segments within these proteins form the core amyloidogenic region that drives fibrillization of the protein, and it has been shown that these segments, alone, form fibrillar structures. Here, we seek to determine the nature of fiber nucleation of these peptides. Amyloid fibers can be nucleated via a fiber-dependent pathway in which additional protein adds onto the ends and extends existing fibers. However, it is hypothesized that the walls of existing fibers can also nucleate amyloid fibers in a peptide-specific manner using the solvent-exposed side chains as a template. Conditions necessary for aggregation have been investigated for a set of amyloidogenic peptides. We have found that aggregation depends on peptide and salt concentration and occurs on the timescale of hours. We have also employed a novel approach for presenting an amyloid fiber wall without presenting fiber ends. These studies illustrate the conditions and geometry important for novel nucleation mechanisms of fiber formation.

259-Pos Board B28

Modulating the Yield But Not Pathway of Protein Self-Assembly by the Surface-Layer Protein SbpA in Solution

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Surface layer (S-layer) proteins form a highly ordered crystalline, yet porous, layer on the outermost cell surface of most species of bacteria and archaea. S-layers not only provide the cell a layer for protection, they are postulated to mediate several functions such as cell adhesion, drug resistance and providing a scaffold for mineralization. Thus, S-layers provide a robust in vitro model system to test our understanding of the dynamics of self-assembly pathways, as well as a biological scaffold for hierarchical assembly of nano-materials. The SbpA protein from Lysinibacillus sphaericus is a well characterized S-layer protein that forms Ca^{2+} dependent, 2D crystals in solution with square symmetry. Here we use ensemble techniques to study the self-assembly process in solution. We use light scattering to follow assembly kinetics and determine a phase a diagram for crystallization; moreover we use electron microscopy and SAXS to confirm that SbpA forms large crystalline sheets in solution, detectable by our assay. Surprisingly, we find that while SbpA requires a minimal amount of Ca^{2+} to crystallize, excess divalent ions in solution inhibit interaction between subunits and thus self-assembly. In addition, we find that the crystallization of SbpA does not require the formation of a nucleus, and that all the steps for self-assembly are energetically favorable. We propose a model in