amino acids could help in driving the desired heterospecificity. Stability and specificity of the desired state was targeted using positive and negative design. With this approach, we have targeted the folding of the heterotrimer state by destabilizing homotrimer states via electrostatic repulsion. In this study, we sought to investigate the specific impact of repulsive interactions at the molecular level. Three peptides have been designed by introducing charge crowded acidic amino acids either at N-terminal, central, or at C-terminal end of the Pro-Hyp-Gly sequence repeats. Our CD and NMR results demonstrate that charge crowding may destabilize the homotrimer states in our triple helical peptide system and can be utilized to promote the formation of the heterotrimer. Increase in salt concentration or decrease in pH result in an increase of homotrimer stability, which confirms the role of charge crowding on the destabilization of homotrimers via electrostatic repulsion. Further investigation is required to understand the molecular role of charge crowding and can be used in conjunction with other approaches to create specific collagen heterotrimers. We further extended our study to create higher order structure by designing basic amino acids sequences in place of acidic amino acids. Mixing of basic and acidic amino sequences resulted in higher order structures.

**3449-Pos Board B177**

**Urea, Guanidine Hydrocloride and 2,2,2-Trifluoroethanol Can Change the Amyloid Fibril Formation of Model Proteins: A Spectroscopic Study**

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The influence of external agents on proteins function and structure is essential to elucidate the unfolding pathways and the protein self-assemble properties. The knowledge of the protein amyloid fibril formation process is important due to the fields that this subjected is related, in particular for the neurodegenerative disorders as Parkinson's and Alzheimer's diseases. In the present study we evidenced the influence of different external agents on the Bovine Serum Albumin (BSA) and Lysozyme amyloid fibril formation by means of UV-Vis and Fluorescence spectroscopy. Concerning BSA, the presence of urea (< 3M) was able to induce the formation of amyloid fibrils at 328 K and increasing urea concentration the amount of protein in the amyloid form also increases. Moreover, a different effect was evidenced by the presence of TFE, where BSA underwent an amorphous aggregation process, leading even to the flocculation of BSA. Interestingly the presence of both urea and TFE up to 5% induces the appearance of amyloid fibrils, instead of amorphous aggregation. Regarding GndHCl, it was not able to induce the formation of amyloid fibrils in neither BSA nor Lysozyme. It is interesting that GndHCl and Urea are well-known as protein denaturant agents, however, their interaction in the protein surface is quite different, such a difference could lead the protein to different final conformations, including the amyloid fibril one. This study indicates that the hydration shell can play an important role in the amyloid aggregation process.

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**3450-Pos Board B178**

**The Oligomeric State of Human Alpha-Defensin 1 in Solution**

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Defensins are small (18-45 amino acids) proteins present in organisms from plants to humans. They function as host defense peptides, assisting the immune system cells in killing phagocytosed bacteria, and are also active against fungi and viruses. The defensins mode of action is membrane permeabilization - forming fatal, pore-like membrane defects in microbial cells. Since membrane permeabilization models require formation of protein dimers or higher oligomers to open pores in the membrane, the defensins propensity to oligomerize is important to their function and is recently a subject of many research projects. Studying oligomerization of small proteins and peptides presents many challenges. Several methods are suitable to detect oligomeric species, especially after chemical cross-linking, but only the light scattering and analytical ultracentrifugation based measurements allow estimating the equilibrium oligomerization constants without chemical modification of proteins. Still, low scattering light intensity, high diffusion and slow sedimentation rates of peptides make obtaining accurate results difficult. We will present a detailed study of the human alpha-defensin 1 (HNP1) oligomerization. HNP1, prevalent in human neutrophil granulocytes, is not only a potent antimicrobial agent, but was also shown to have cytotoxic activity against many mammalian tumor cells in vitro.

**3451-Pos Board B179**

**Aggregation of Transforming Growth Factor β Induced Protein Studied by Protein-Protein Docking**

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Transforming growth factor β induced protein (TGFBIp) is an extracellular matrix protein that has been linked to several types of corneal dystrophies. Nearly fifty percent of the mutations leading to corneal dystrophies affect two major single residue hotspots, namely R124 and R555, located in the first and the fourth FAS1 like domain of TGFBIp, respectively (1). The remaining mutations are primarily located in the fourth FAS1 like domain (FAS1-4). Three mutations in the FAS1-4 domain of TGFBIp are investigated in the present study: R555W, leading to granular corneal dystrophy; R555Q, leading to Thiel-Behnke corneal dystrophy; and A546T, leading to lattice corneal dystrophy. Of the three mutations, A546T is the only one resulting in deposits of amyloid fibrils *in vivo* and *in vitro.* The aim of this study is to investigate whether or not the three pathogenic mutations give rise to distinct association poses discerning between amorphous and amyloid phenotypes. Protein-Protein docking calculations have been performed using the docking algorithm DOCK/PIERR (2). Two highly abundant poses involving direct contact between the suspected fibrillating core regions of the FAS1-4 domain have been identified. Runager et al. has shown the A546T mutant to be the least stable of the three mutants (3). Combining the decreased stability with the docking poses could give an explanation to the amyloidogenic nature of the A546T mutant.

1. Munier et al. *Invest. Ophthalmol. Vis. Sci.* **2002***, 4,* 949-954.
2. Ravikant et al. *Proteins* **2010***, 2,* 400-419.
3. Runager et al. *J. Biol. Chem* **2011***, 7,* 4951-4958.

**3452-Pos Board B180**

**Stable Amyloid Oligomers can Seed Fibril Growth Near Physiological Conditions**

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Spontaneous formation and deposition of protein fibrils with cross beta-sheet structure are associated with an increasing number of human disorders including Alzheimer's disease, senile systemic or dialysis-related amyloidosis. The morphological and structural similarities of amyloid deposits and their various intermediates suggest that amyloid diseases share basic aspects of their etiology. Elucidating the molecular mechanisms underlying these similarities has been challenging since biophysical studies of fibril formation *in vitro* require partial denaturation of native proteins. These conditions are far from the physiological environment relevant to fibril growth *in vitro*. Here we show that metastable amyloid oligomers and protofibrils of lysozyme, transiently formed under denaturing conditions, can become stabilized at physiological conditions. In addition, they are capable of inducing the conversion of natively folded, monomeric lysozyme into aggregated β-sheet rich fibrils *in vitro*. There was no discernible threshold concentration of lysozyme monomers below which seeding didn't induce growth.

Our data suggest that only the nucleation of amyloid oligomers is a low-probability event requiring partially denaturing conditions. Once formed, these seeds can become stabilized under near physiological conditions and induce seed elongation and fragmentation. The stabilization of amyloidogenic intermediates away from nucleation conditions, their ability to remain soluble or to seed subsequent fibril growth of native monomers appear to be protein-specific. The oligomer/protofibril-induced growth of native lysozyme observed in our experiments bears intriguing similarities with the ability of prion proteins to drive the autocatalytic conversion of their native counterpart into amyloid fibrils.

**3453-Pos Board B181**

**Phase Boundaries for Fibril and Metastable Oligomer Formation of Lysozyme**

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Deposition of protein fibers with a characteristic cross-beta sheet structure is the molecular marker for many human disorders including Alzheimer's disease, type II diabetes and rheumatoid arthritis. Given the large number of proteins and peptides beyond those associated with diseases that have been shown to form amyloid fibrils in vitro, it has been suggested that amyloid fibril formation represents a generic protein phase transition. Mapping out the corresponding phase boundaries is complicated by the presence of least two distinct fibril assembly pathways. One pathway is characterized by the nucleation of long, rigid fibrils common to the late stages of amyloid diseases. A second pathway involves the formation of globular oligomeric species and curvilinear protofibril.

The relation of this latter pathway to the mature fibrils is not entirely clear. Intriguingly, it is these latter oligomers and protofibrils that have been implicated as the molecular species mediating the cellular toxicity associated with amyloid diseases.
For the amyloidogenic protein lysozyme, we have systematically mapped out the combination of protein and salt concentrations resulting in the formation of either long rigid or oligomeric amyloid aggregates for fixed temperature and pH. Using dynamic light scattering, thioflavin fluorescence spectroscopy, atomic force microscopy and infrared spectroscopy, we detected three distinct types of aggregates. Growth of long straight fibrils prevailed at either low salt or protein concentrations. At intermediate salt and protein concentration oligomer formation with subsequent protofibril nucleation prevailed. Oligomers and protofibrils represent metastable phases that are kinetically favored, while long straight fibrils are the thermodynamically stable state. Eventually, fibril formation gives way to amorphous precipitation. This phase behavior shows intriguing similarities with the phase diagram for protein crystallization where a metastable liquid-liquid phase is located within the stable coexistence region for protein crystals.

**3454-Pos Board B182**
**Polyglutamine Flanking Regions in Huntingtin Highlight Key Structural Intermediates Relevant for Molecular Chaperone Interaction and Huntington's Disease Pathogenesis**
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Huntington's Disease (HD) is a neurodegenerative disorder caused by an expansion in the polyglutamine (polyQ) tract of the Huntingtin (Htt) protein. The clinical hallmark of this mutation is the accumulation of amyloid Htt aggregates in neurons. While Htt aggregation is highly correlated with the length of this polyQ tract both in vitro and in vivo, recent studies suggest that the regions flanking the polyQ tract can influence Htt aggregation and toxicity independent of polyQ length. In addition, it is well established that two polyQ flanking regions in exon1 of Htt, the N-terminal first 17 amino acids (N17) and a proline-rich region (PolyPro), influence Htt aggregation propensity. Here, we show that the N17 and PolyPro regions of Htt dramatically influence the rate of the Htt aggregation pathway. We also show that mutations within these polyQ-flanking regions alter Htt toxicity in a brain slice model. The influence of these flanking regions on the heterogeneous distribution of the aggregate species may account for these differences in toxicity. This theory is increasingly relevant in light of recent thought that Htt toxicity may derive from a species other than the amyloid fibril itself. Finally, previous work has shown that the N17 region of Htt interacts with the molecular chaperonin TRiC, and this interaction exerts a protective effect against both Htt aggregation and toxicity. An understanding how these flanking regions influence Htt aggregation will inform how TRiC works through the N17 element. Together, this information can provide the basis to design HD therapeutics that exploit these flanking regions to suppress HD pathogenesis.

**3455-Pos Board B183**
**Kinetics of the Interconversion Between Two Physiologically Important Copper-Bound Amyloid-Beta Species**
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To better understand the physiological behaviour of Aβ in the brain, the kinetics of the interactions between metal ions and Aβ is crucial. In 1:1 stoichiometry binding between cooper ions and Aβ, two major conformations have been characterized in equilibrium in literature. We found that Aβ binds to a first copper ion with a near diffusion limited rate constant ~10 ^ 8 M ^ {-1}s ^ {-1}, to a second copper ion at a rate constant ~10 ^ 5 M ^ {-1}s ^ {-1}, and further copper ions. The reaction between EDTA and a mixture of 50 nM Aβ and various amounts of copper ions ranging from nM to 10 μM shows the evolution of multiple copper-Aβ species as a function of copper concentration. Most interestingly, the ratio of the two major species at low copper concentrations depends on EDTA concentration, suggesting the interconversion between them, with the rates in the order of s ^ {-1}. Whether this interconversion is relevant to the roles of Aβ in health and disease is unclear.

**3456-Pos Board B184**
**Insights into the Inhibition Mechanism of Biomolecular Self-Assembly from Chemical Kinetics**
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Understanding and control the aggregation of biomolecules at the molecular level can open attractive possibilities to correct dysfunctional cell behaviour. For instance, the inhibition of protein aggregation is emerging as a potential attractive therapeutic strategy against several neurodegenerative disorders. For the development of successful treatments, it is crucial to achieve a controlled intervention on specific toxic species. In this perspective, an understanding of the molecular inhibition mechanism of protein self-assembly is of fundamental importance but remains challenging to achieve.
In this work, we demonstrate how chemical kinetic analysis can be applied to elucidate the molecular mechanism of inhibition of several classes of compounds such as small chemical molecules, nanoparticles, peptides and proteins. By applying a population balance model we show how it is possible to obtain information on the specific inhibited microscopic event and on the specific protein target species responsible for this inhibition. We demonstrate the potentiality of the approach by analyzing the inhibition mechanism of selected chaperones, protein regulators of the proteostais network and relevant naturally occurring inhibitors of protein aggregation, on the aggregation of a yeast prion protein and of Abeta42, the peptide involved in Alzheimer's disease. In addition, we discuss relevant implications of the controlled inhibition of protein aggregation in the engineering of the fibrillation reaction pathway and in the development of effective therapeutic strategies.

**3457-Pos Board B185**
**Filament Assembly by Phosphofructokinase-1, the Gatekeeper of Glycolysis**
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The cytoskeleton is conventionally viewed as being composed of three filamentous networks; microfilaments, microtubules, and intermediate filaments. This view is challenged by the findings that metabolic enzymes can form filaments with structural functions. We report that phosphofructokinase-1 (PFK1), the first rate-limiting step of glycolysis, assembles into filaments in vitro and in cells. Transmission electron microscopy (TEM) showed that purified liver PFK1 is mainly tetrameric and occasionally formed short filaments in the absence of substrate. Adding the substrate fructose 6-phosphate (F6P) induced the assembly of predominantly long filaments measuring up to 250 nm. PFK1 filaments were less rigid than actin polymers, displaying right angles in contiguous assemblies. The filaments were composed of individual tetramers and had a uniform 11 nm width, resembling an organized addition of subunits forming polymers. Regulated assembly into filaments was also indicated by light scattering measurements that showed a rapid substrate-dependent increase in scattering followed by a stable plateau. Increased light scattering was blocked by excess ATP, which inhibits PFK1 activity. To further confirm activity-dependent filament assembly we generated an inactive but tetrameric liver PFK1 mutant, His199Tyr, and found that in the presence of F6P it does not form filaments, as determined by TEM, or show an increase in light scattering. To assess filament formation by PFK1 in cells, we expressed GFP-tagged PFK1 and used live-cell imaging to examine GFP-PFK1 dynamics. Confocal microscopy reveled that cytosolic PFK1 was recruited to the distal margin of lamellipodia that were devoid of mitochondria. TIRF microscopy reveled that GFP-PFK1 formed dynamic punctae. These data indicate that active but not inactive PFK1 assembles into tetramer-aligned filaments. The activity-dependent recruitment and assembly of PFK1 filaments at the plasma membrane could provide a scaffolding and structural framework for localized ATP production in lamellipodia that lack mitochondria.

**3458-Pos Board B186**
**Amyloid β-Protein: The Influence of Intrinsic and Extrinsic Factors on Fibril Formation**
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Aggregation of the amyloid β-protein (Aβ) is believed to be involved in Alzheimer's disease pathogenesis. The central hydrophobic region (CHR) and the Aβ42/Aβ40 ratio play key roles in Aβ aggregation. Studying intrinsic (amino acid substitutions) and extrinsic (temperature, other molecules) factors contributes to understanding the mechanisms that cause Aβ monomers to aggregate and form oligomers and fibrils. This could facilitate the development of agents that therapeutically target toxic assemblies or prevent their formation.
In our studies we mainly used a highly reproducible thioflavin T assay to probe the aggregation kinetics. Substitution of phenylalanine with leucine at position