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Organizing committee:

Daniel Otzen (Aarhus), Poul Henning Jensen (Aarhus), Sven Frøkjær (Copenhagen) & Niels Borregaard (Copenhagen)

Abstracts - MONDAY, August 24, 2009

AMYLOID DISEASES - AN HISTORIC PERSPECTIVE FROM CRUDE MASS TO SOLUBLE PROTOFIBRILS

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My colleagues and I started work on purifying the amyloid plaques of Alzheimer's disease (AD) and Creutzfeldt-Jakob disease (CJD) in 1978. We found that the AD Aβ amyloid was derived from neurons by proteolytic processing of a transmembrane precursor protein (APP), which has led to the development of the Aβ theory of AD.
Compelling evidence now shows that the Aβ-amyloid peptide is the central biochemical marker of AD, and is the most likely cause of the neurodegeneration manifest in synaptic dysfunction and eventual neuronal loss. Pathways up-stream of Aβ production provides therapeutic targets amenable to protease inhibition/modulation. Strategies which affect APP trafficking may also prove of value. Downstream, pathways promoting the degradation of Aβ or modulating clearance from the brain also offer windows for therapeutic opportunity.
Central interest lies in the mechanism through which Aβ undergoes toxic gain-of-function, inducing neuronal damage. This provides the most direct route for therapeutic intervention, with least risk of therapeutic side-effects, since Aβ toxicity is unlikely to mimic any normal function. Two principal hypotheses have emerged to explain Aβ toxicity: redox chemistry associated with the Cu/Zn metal binding sites on Aβ, and lipid interactions associated with the α/β conformation of the hydrophobic C-terminus. Drugs targeting these mechanisms are now in clinical development with encouraging preliminary results.
The normal function of APP remains elusive despite two decades of research. Dimerization of APP through the transmembrane domain and other regions leads to proteolytic release and signaling through its cytoplasmic domain. Perturbation of processing may lead to excessive production of Aβ peptide. Either as a soluble oligomer or insoluble amyloid fibril, the accumulation of the Aβ fragment provides a pivotal biomarker, currently being developed as a neuroimaging target and a blood/CSF biomarker for efficacy of therapeutic intervention, and for gene-linkage discovery.

FUNDAMENTAL QUESTIONS ABOUT AMYLOIDS AND PRIONS, FIBRIL-FORMING PROTEINS ASSOCIATED WITH DEGENERATIVE DISEASES

Eisenberg D, Sawaya M, Nelson R, Goldschmidt L, Sievers S, Sambashivan S, Ivanova M, Landau M, Apostol M, Teng P, Zhao M, Balbirnie M, Cheng H, Zhao A, MacFarlan H, Cascio D, Gingery M & Park J; Departments of Biological Chemistry and Chemistry & Biochemistry, UCLA-DOE Institute for Genomics & Proteomics, USA

Coordinated structural, biochemical, and computational studies have answered several fundamental questions at the molecular level about amyloids and prions. These include:

- What is the atomic structure of the fibrillar spine?
- Does amyloid formation depend on amino acid sequence?
- Why do dissimilar proteins form similar fibrils?
- During fibrillation, what happens to protein segments not included in the spine?
- Which proteins can form amyloids (the "amylome")?
- What is molecular basis of prion strains? What causes the lag time prior to fibrillation?
- Can amyloid fibrils be inhibited?

References:

1. "Atomic structures of amyloid cross-beta spines reveal varied steric zippers" Sawaya et al. Nature, 447, 453-457 (2007).
2. "Amyloid-like fibrils of ribonuclease A with three-dimensional domain-swapped and native-like structure" Sambashivan et al. Nature 437, 266-269 (2005).
3. "The 3D profile method for identifying fibril-forming segments of proteins." Thompson et al. Proc. Natl. Acad. Sci. USA 101, 4074-4078 (2006).

THE ARCHITECTURE OF THE CROSS-BETA FOLD FOR AMYLOID

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Amyloid fibrils assemble from a vast range of different large proteins and short peptides, with diverse primary sequences, and yet the structures of the mature amyloid fibrils show remarkable similarities. The resulting mature fibrils are highly ordered and have been generally represented by the cross-beta structure in which beta-strands run perpendicular to the fibre axis and are hydrogen bonded along the length of the fibre to create highly stable, organised protofilaments. Recent advances in X-ray crystallography have supported this view of the amyloid architecture and structures arising from solid state NMR have been based on cross-b like arrangements. Electron microscopy and cryo-electron microscopy have shown that amyloid fibril can vary at the macromolecular level, but generally mature fibrils are formed by the association of individual protofilaments. Here, we will discuss the essential elements of the cross-b structure, the evidence for this structure that comes from X-ray fibre diffraction and available insights into the importance of side chains composition and their contribution to the stability of the overall structure. In particular, the role of aromatic and hydrophobic residues has been examined in depth using short model systems to investigate the packing between beta sheet ribbons.

PREDICTING PROTEIN BEHAVIOUR FROM PHYSICO-CHEMICAL PRINCIPLES

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Stringent conditions on the physico-chemical properties of proteins, and hence of the nature of their amino acids, are imposed by the need to avoid aggregation at the concentrations required for optimal cellular function [1,2]. A relationship is therefore expected to exist between mRNA expression levels and protein solubility in the cell. By investigating such a relationship we show that it is possible to predict the maximal

levels of mRNA expression in E. coli with an accuracy of 83%, and of the solubility of recombinant human proteins expressed in E. coli with an accuracy of 86%.

References:
1. G. G. Tartaglia, S. Pechmann, C. M. Dobson and M. Vendruscolo; Life on the edge: A link between gene expression levels and aggregation rates of human proteins, Trends Biochem. Sci. 32, 204-206 (2007).
2. G. G. Tartaglia, S. Pechmann, C. M. Dobson and M. Vendruscolo; A relationship between mRNA expression levels and protein solubility in E. coli, J. Mol. Biol. in press (2009).

TOWARDS A FULL DESCRIPTION OF THE POLYMORPHIC FIBRILLATION OF GLUCAGON

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The 29-residue peptide hormone glucagon exhibits an amazing versatility in its fibrillation behaviour, which is highly sensitive to experimental conditions, such as temperature, pH and concentration. This indicates the availability of a multitude of different packing arrangements that lead to fibrils of different structures and stabilities. We have studied the properties of these fibrils, as well as the pathways leading to them, using a very broad range of biophysical techniques, including fiber diffraction, isothermal titration calorimetry, Small Angle X-ray Scattering and Quartz Crystal Microbalance with Dissipation . In my talk I will attempt to provide a scheme that provides a uniform view of these processes.

References:
1. Pedersen, J. S., Dikov, D., Flink, J. L., Hjuler, H. A., Christiansen, G., & Otzen, D. E. (2006) *J. Mol. Biol.***355,** 501-523.
2. Pedersen, J. S., Dikov, D., Flink, J. L., & Otzen, D. E. (2006) *Biophys. J.* **90,** 4181-4194.
3. Pedersen, J. S., Dikov, D., & Otzen, D. E. (2006) *Biochemistry* **45,** 14503-14512.
4. Pedersen, J. S. & Otzen, D. E. (2008) *Protein Science* **17,** 1-9.
5. Christensen, P. A., Pedersen, J. S., Christiansen, G., & Otzen, D. E. (2008) *FEBS Lett.* **582,** 1341-1345.
6. Oliveira, C. L. P., Behrens, M. A., Pedersen, J. S., Erlacher, K., Otzen, D. E., & Pedersen, J. S. (2009) *J. Mol. Biol.* **In press**.
7. Dong, M., Hovgaard, M. B., Xu, S., Otzen, D. E., & Besenbacher, F. (2006) *Nanotechnology* **17,** 1-6.
8. Hovgaard, M. B., Dong, M., Otzen, D. E., & Besenbacher, F. (2007) *Biophys. J.* **93,** 2162-2169.
9. Dong, M., Hovgaard, M. B., Mamdouh, W., Xu, S., Otzen, D. E., & Besenbacher, F. (2008) *Nanotechnology* **19,** 384013.
10. Andersen, C. B., Otzen, D. E., Christiansen, G., & Rischel, C. (2007) *Biochemistry* **46,** 7314-7324.
11. Svane, A. S. P., Jahn, K., Deva, T., Malmendal, A., Otzen, D. E., Dittmer, J., & Nielsen, N. C. (2008) *Biophys. J.* **95,** 366-377.
12. Andersen, C. B., Yagi, H., Manno, M., Martorana, V., Ban, T., Christiansen, G., Otzen, D. E., Goto, Y., & Rischel, C. (2009) *Biophys. J.* **96,** 1529-1536.

A THERAPEUTIC APPROACH TO PROTEIN AGGREGATION DISEASES

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This talk will report on a novel therapeutic approach to promoting clearance of cytosolic and potentially pathogenic protein aggregates. The historical context of this approach will be discussed, beginning with the identification of a novel substrate for protein farnesylation, UCH-L1 (Z Liu, et al PNAS 2009). The effects of a candidate therapeutic in mouse models of neurodegeneration will be discussed, along with studies in cell culture that suggest a mechanism of action. In addition to the

underlying science, the path leading from these studies to clinical trials will be discussed.

FOLLOWING OLIGOMER AND AMYLOID FORMATION BY SMALL ANGLE X-RAY SCATTERING

Vestergaard B[1,2], Groenning M[2], Giehm L[3], Langkilde AE[1], Frøkjær S[2], Otzen D[3], & Svergun DI[4]; [1]Dept. Medicinal Chemistry and [2]Dept. Pharmaceutics and Analytical Chemistry, Faculty of Pharmacuetical Sciences, University of Copenhagen, Denmark, [3]Interdisciplinary Nanoscience Centre, University of Aarhus, Denmark, [4]EMBL-Hamburg, Germany

Small-angle X-ray scattering (SAXS) offers unique means for structural analysis of fibril formation. Individual species, present in equilibrium in solution, may be modelled based on data on the evolving fibrillation, hence equilibriums between species are left undisturbed while measuring. Using this approach, we have analysed several fibrillating protein systems. For the first time, the solution structure of an on-pathway oligomeric species, which - as it will be outlined - represents the structural nucleus, has been determined. Results will be presented from both published and unpublished data from human insulin, α–synuclein and selected peptide systems under varying experimental conditions. The results will be correlated with existing theories of fibril elongation, and a plausible model of elongation using the structural nucleus as a building block will be presented. Finally, the option that such an oligomeric species represents the (most) cytotoxic component during the reaction will be debated in context of recent experimental evidence.

References:

1. *A helical structural nucleus is the primary elongating unit of insulin amyloid fibrils.* Vestergaard, B., Groenning, M., Roessle, M., Kastrup, J.S., van de Weert, M., Flink, J.M., Frokjaer, S., Gajhede, M., Svergun, D.I. (2007) PLoS Biology, 5: 134

Poster No. I-1

QUARTZ CRYSTAL MICRO-BALANCES (QCM) FOR ACCURATE KINETIC MEASUREMENTS OF AMYLOID GROWTH

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Precise and reliable measurements of the kinetics of amyloid fibril growth are difficult to perform. In order to extract the maximum amount of information about the misfolding/folding free energy surface of the protein from these kinetic measurements, it is necessary to correlate the measured signal with the number of growing fibrils. This is very challenging in bulk solution measurements. Surface bound measurement techniques; however present the unique opportunity to determine the number of growing fibril ends via imaging of the surface after an experiment. We present a recently developed method [1], using quartz crystal micro-balances. Here, the growth of surface bound amyloid fibrils is monitored in real time via their increase in mass. Due to the possibility to repeatedly probe the growth of the same ensemble of surface-bound fibrils, excellent reproducibility is achieved. This feature also provides the necessary sensitivity to detect subtle changes in aggregation rate stemming from changes in external conditions and amino acid sequence. Examples of measurements are presented where the temperature dependence of the aggregation rate of different fibril forming proteins is compared. This experiment allows for the determination of the enthalpic part of the free energy

barrier that a protein monomer has to overcome in order to incorporate into a fibril. Together with a suitable kinetic model, this also enables the determination of the entropic contribution to the free energy barrier which has proven very difficult with any other technique available to date. In addition, results of a mutational study [2] are presented where the influence on the aggregation rate of addition of a charged amino acid at different positions in the sequence of an amyloidogenic SH3 domain are shown. This is the first fully quantitative assessment of the "gate keeper" effect.

References:

1. Tuomas P. J. Knowles, Wenmiao Shu, Glyn L. Devlin, Sarah Meehan, Stefan Auer, Christopher M. Dobson, and Mark E. Welland, PNAS, *2007, 104, 10016-10021*
2. Alexander K. Buell, Gian Gaetano Tartaglia, Neil R. Birkett, Christopher A. Waudby, Michele Vendruscolo, Xavier Salvatella, Mark E. Welland, Christopher M. Dobson, and Tuomas P.J. Knowles, *ChemBioChem, accepted for publication*

Poster No. I-2

LIPID BINDING PROPERTIES OF OLIGOMERIC α–SYNUCLEIN

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Oligomeric aggregates of α–synuclein have been implicated to play a central role in the pathogenesis of Parkinson's disease. Disruption and permeabilization of lipid bilayers by α–synuclein oligomers is postulated as a toxic mechanism, but the molecular details controlling the oligomer-membrane interaction are still unknown. We have systematically studied the influence of the physical membrane properties and solution conditions on lipid bilayer disruption by oligomeric α–synuclein using a dye release assay. Furthermore, we have quantitatively studied oligomer lipid binding by confocal fluorescence microscopy and fluorescence correlation spectroscopy (FCS). The results indicate that the oligomeric species specifically bind to negatively charged lipids in the liquid disordered phase. The lipid binding affinity of oligomeric α–synuclein for DOPS vesicles is around two times lower compared to monomeric α–synuclein. The binding specificity for negatively charged lipids implicates that the α–synuclein oligomers have defined structural features and are not merely amorphous protein aggregates. As expected from the binding properties, membrane disruption only occurs in vesicles composed of negatively charged lipids and depends on lipid bilayer packing parameters. More densely packed membranes are less sensitive to oligomer induced membrane permeabilization. Comparing the concentration dependence of oligomer lipid binding and oligomer vesicle disruption for different lipid compositions revealed that lipid bound oligomers do not cause membrane disruption under all conditions. The actual lipid composition determines if membrane bound oligomers lead to membrane permeabilization.

Poster No. I-3

BRANCHING IN AMYLOID FIBRIL GROWTH

Andersen CB[1,3], Yagi H[2], Manno M[3], Martorana V[3], Ban T[2], Christiansen G[4], Otzen D[5], Goto Y[2] & Rischel C[1]; [1]Protein Structure and Biophysics, Novo Nordisk, Denmark, [2]Inst. for Protein Research, University of Osaka, Japan, [3]Institute of Biophysics, CNR Palermo, Italy, [4]Institute of Medical Microbiology and Immunology, University of Århus, Denmark, [5]Interdisciplinary Nanoscience Centre, University of Aarhus, Denmark

Using the peptide hormone glucagon and $A\beta(1–40)$ as model systems, we have sought to elucidate the mechanisms by which fibrils grow and multiply [1]. We here present real-time observations of growing fibrils at a single-fibril level. Growing from preformed seeds, glucagon fibrils were able to generate new fibril ends by continuously branching into new fibrils. To our knowledge, this is the first time amyloid fibril branching has been observed in real-time. Glucagon fibrils formed by branching always grew in the forward direction of the parent fibril with a preferred angle of 35–40. Furthermore, branching never occurred at the tip of the parent fibril. In contrast, in a previous study by some of us, $A\beta(1–40)$ fibrils grew exclusively by elongation of preformed seeds. Fibrillation kinetics in bulk solution was characterized by light scattering. A growth process with branching, or other processes that generate new ends from existing fibrils, should theoretically give rise to different fibrillation kinetics than growth without such a process. We show that the effect of adding seeds should be particularly different in the two cases. Our light-scattering data on glucagon and $A\beta(1–40)$ confirm this theoretical prediction, demonstrating the central role of fibril-dependent nucleation in amyloid fibril growth.

References:

1. Andersen CB, Yagi H, Manno M, Martorana V, Ban T, Christiansen G, Otzen DE, Goto Y & Rischel C (2009) Branching in amyloid fibril growth. *Biophys. J.* **96**:1529-1536.

Poster No. I-4

SWITCH OF AMYLOID STRAIN WITHIN INDIVIDUAL AMYLOID FIBRILS

Baskakov IV[1], Makarava N[1], Ostapchenko VG[1], Shashilov V[2], Xu M[2], Savtchenko R[1] & Lednev, IK[2]; [1]Medical Biotechnology Center, University of Maryland, USA, [2]Department of Chemistry, University of Albany, USA

A key structural component of amyloid fibrils is a highly-ordered, crystalline-like cross β-sheet core. Conformationally different self-propagating amyloid structures referred to as amyloid strains can be formed within the same amino acid sequence. It is generally assumed that individual fibrils consist of conformationally uniform cross b-structures. Using mammalian prion protein (PrP), single-fibril microscopy combined with atomic force microscopy and hydrogen-deuterium exchange Raman spectroscopy, we showed that contrary to common perception, amyloid is capable of accommodating a significant conformational change within individual fibrils. The conformational change occurred when the amino acid sequence of a PrP variant used as a precursor substrate in a fibrillation reaction was not compatible with the strain-specific conformation of the fibrillar template. Despite the mismatch in amino acid sequences between substrate and template, individual fibrils recruited the heterologous PrP variant; however, the fibril elongation proceeded through a conformational adaptation resulting in a change in amyloid strain within individual fibrils. The change in amyloid strain was unidirectional, i.e. the species-specific amyloid strain converted into promiscuous one, but not vice versa.

This study illustrates the high adaptation potential of amyloid structures and suggests that conformational switching within individual fibrils may account for adaptation of amyloid strains to a heterologous substrate. The conformational adaptation occurred in one direction: from species-specific amyloid structures to indiscriminative or promiscuous ones but not vice versa. The current work proposes a new mechanistic explanation for the phenomenon of strain conversion and illustrates the direction in evolution of amyloid structures. This study also provides a direct illustration that catalytic activity of self-replicating amyloid structures are not ultimately coupled with their templating effect.

Poster No. I-5

A BETA-2 MICROGLOBULIN VARIANT FIBRILLATES AT NEAR-PHYSIOLOGICAL PH

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β_2-microglobulin (β_2m) deposits as amyloid in dialysis related amyloidosis (DRA), predominantly in joints. The molecular mechanisms underlying the amyloidogenicity of β_2m are still largely unknown. *In vitro,* acidic conditions, pH <4.5, induce amyloid fibrillation of native β_2m within several days. Here, we show that fibrils having amyloid characteristics, are generated in less than an hour when a cleavage variant of β_2m (DK58-β_2m) – found in the circulation of many dialysis patients [1] – is exposed to pH-levels occurring in joints during inflammation (pH ~6.8). Aggregation and fibrillation, including seeding effects with intact, native β_2m were here studied by Thioflavin T fluorescence spectroscopy, turbidimetry, capillary electrophoresis and electron microscopy. In conclusion, our results show a high fibrillation propensity of a biologically relevant β_2m-variant, resulting in fibrils having the ability to seed subsequent generation of fibrils from native β_2m. These observations may be useful in clarifying the initiation of dialysis related amyloidosis.

References:

1. Corlin DB, Sen JW, Ladefoged S, Lund GB, Nissen MH, & Heegaard NHH (2005) Quantification of Cleaved {beta}2-Microglobulin in Serum from Patients Undergoing Chronic Hemodialysis. Clin. Chem., 51, 1177-1184

Poster No. I-6

PHOSPHOLIPID-INDUCED FIBRILLATION OF A PRION AMYLOIDOGENIC DETERMINANT AT THE AIR-WATER INTERFACE.

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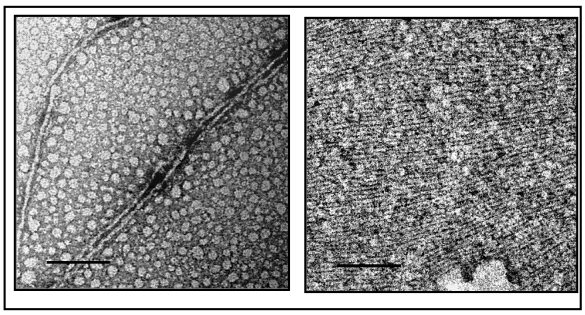
The peptide fragment 106-126 of prion protein [PrP(106-126)] is a prominent amyloidogenic determinant.

We present analysis of PrP(106-126) fibrillation at the air/water interface and, in particular, the relationship between the fibrillation process and interactions of the peptide with phospholipid monolayers. We find that anionic phospholipid monolayers deposited at the air/water interface induce rapid formation of remarkably highly-ordered amyloid fibrils by PrP(106-126). The extent of fibrillation and fiber organization were dependent upon the content of negatively-charged phospholipids in the monolayers and the surface pressure of the film. We also observe that fibrillation was enhanced when PrP(106-126) was injected underneath pre-assembled phospholipid



TEM image of fibrils formed by PrP(106-126) at the air/water interface. A) In water; B) In negatively charged monolayer.

monolayers, compared to deposition and subsequent compression of peptide/phospholipid mixed films at the air-water interface.
In a broader context, this study demonstrates that Langmuir systems provide a useful platform for studying lipid interactions of amyloidogenic peptides and lipid-induced fibrillation phenomena.

Poster No. I-7

NUCLEATION MECHANISMS, MORPHOLOGIES AND DETECTION IN INSULIN FIBRILLATION

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At low pH and high temperature insulin is highly prone to self-assembly into amyloid fibrils. Firstly, our results on human insulin fibrillation in acetic acid at 45°C suggest that the overall fibrillation kinetics are mainly governed by the accessible surface, through secondary nucleation mechanisms [1]. Moreover, a statistical study of the fibrillation kinetics suggests that the early stages of the process are affected by stochastic nucleation events [1]. Further, we also focused on the aggregation mechanisms of bovine insulin occurring in HCl solutions (pH 1.6) at 60°C. Using a Thioflavin T (ThT) assay, two concurrent aggregation pathways have been pointed out with different morphologies of self-assembled protein molecules as detected by atomic force microscopy images. Moreover, combining ThT fluorescence and dynamic light scattering, the early stages of the process have been analyzed in the low concentration regime and a pronounced spatial heterogeneity in the formation of the first stable fibrils in solution has been revealed [2]. Finally, spectroscopic properties and the chemical stability of ThT in aqueous solution have been determined [3]. A reversible hydroxylation process occurs in alkaline solutions and, mainly based on optical spectroscopic studies, we propose a chemical structure for the hydroxylated form. Finally, by means of fluorescence spectroscopy, ThT hydroxylation effects on in situ amyloid detection have been investigated, providing new insights into the efficiency of the ThT assay for quantitative fibril evaluation at basic pH.

References:
1. Foderà V. et al 2008 J Phys Chem B 112, 3853-3858.
2. Librizzi F. et al 2007 Eur Biophys J 36(7), 711-15.
3. Foderà V. et al 2008 J Phys Chem B 112 (47) 15174-15181

Poster No. I-8

LIPID MEMBRANES INTERFER WITH AMYLOID AGGREGATION AND CAN INDUCE FIBRIL DEGRADATION: LESSONS FROM COMPUTER SIMULATIONS

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Accumulating evidence suggests that toxicity in amyloid-related diseases originates from the deposition of protein aggregates on the cell membrane, which results in bilayer disruption and cell leakage. Interestingly, it has also been shown that interactions with certain types of lipids may prevent fibril formation and even induce fibril degradation. To shed light on the molecular mechanism of the interactions between lipids and amyloid peptides, we have performed coarse-grained molecular dynamics simulations of fibril-forming amphipathic peptides in the presence of lipid vesicles (Friedman *et al*., JMB, in press). The simulation results show that highly

amyloidogenic peptides fibrillate on the surface of the vesicle, damaging the bilayer and promoting leakage. In contrast, the ordered aggregation of peptides with low amyloidogenicity is hindered by the vesicles. Remarkably, leakage from the vesicles is caused by growing aggregates, but not mature fibrils. Fibril degradation is induced by lipids that strongly interact with peptide monomers, and depends on the morphology of the fibrils. Taken together, the simulation results provide a basis for understanding the range of aggregation behaviour that is observed in experiments with fibril-forming (poly)peptides.

Poster No. I-9

STUDYING GLUCAGON FIBRILLATION IN SOLUTION

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Protein fibrillation and aggregation is associated with many debilitating diseases. We have been studying a fibrillating peptide glucagon, as a simple model system to understand the fibrillation mechanism in detail. It has the propensity to form polymorphic fibrils at different fibrillation conditions [1]. The differences in the fibril morphology, stability and secondary structure could be used to probe the mechanisms that explain this fibrillar polymorphism. It has been indicated that fibrils formed at unfavourable conditions exhibit greater stability than those formed under ambient conditions of fibrillation [2]. Precise analysis on the secondary structural changes and thermal stability; followed via circular dichroism, fourier transform infrared spectroscopy and other techniques; would give information about how the fibrils rearrange their structure to meet the changing environment (e.g. higher temperatures, pH or high hydrostatic pressures). One of the aims is to systematically analyze the changes in the apparent stability of fibrils with temperature. Fibril imaging techniques like atomic force microscope would also be used to visualize the fibrillar morphology. We hope to extend the knowledge obtained on the fibrillation pathways and mechanisms through our model peptide to other proteins that are prone to cause aggregation / fibrillation disorders.

References:

1. Pedersen J. S., et al (2006) The changing face of glucagon fibrillation: Structural polymorphism and conformational imprinting. *J. Mol. Bio* **355**, 501-523.
2. Pedersen J. S., and D. E. Otzen (2007) Amyloid a state in many guises: Survival of the fittest fibril fold. *Prot. Sci.* **17**, 2-10.

Poster No. I-10

ON THE ROLE OF METAL IONS AS MODULATORS OF PROTEIN AGGREGATION: THE POTENTIAL INVOLVEMENT OF ZINC IN SOD1 PATHOLOGICAL AGGREGATION EVENTS

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Cu/Zn superoxide dismutase (SOD1) is a dimeric protein implicated in amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disorder. SOD1 aggregation results in a toxic gain-of-function, and although mutations and adverse physiological factors increase the oligomerization propensity, even normal SOD1 can aggregate under physiological conditions. It is well established that SOD1 metallation enhances significantly the protein stability and it has been shown that binding of a single zinc

ion to the apoSOD1 dimer significantly influences the structure and stability of the entire protein [1]. In this respect, we are investigating the relationship between zinc-induced SOD1 structural alterations and the formation of aggregates, using biophysical methods. Here we will report our results suggesting that at increasing concentrations of zinc, apoSOD1 undergoes particular conformational alterations and that this is followed by a significant increase in the propensity to form non-native oligomeric species, as suggested by DLS. This effect is accompanyed by a significant change in the relative content in secondary structure of SOD1, noticeably an increment of the intermolecular β-sheet aggregation band, as observed by FT-IR. These observations illustrate how even a transient imbalance in cellular zinc concentrations may trigger the formation of toxic SOD1 aggregates, and our results will also be discussed in the context of physiological zinc bursts, such as those occuring in synaptic signalling.

References:
1. Potter SZ et al JACS 2006 129, 4575-4583.

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Poster No. I-11

STRUCTURAL CHARACTERIZATION OF TRANSTHYRETIN OLIGOMERS

Groenning M[1,2,3], Vestergaard B[1,2], van de Weert M[1], Frøkjær S[1] & Hammarström P[3]; [1]Department of Pharmaceutical Sciences and [2]Department of Medicinal Chemistry, University of Copenhagen, Denmark, [3]Department of Physics, Chemistry and Biology, Linköping University, Sweden

Several studies have shown that the early intermediates in the fibril formation process rather than the amyloid fibrils themselves are the most cytotoxic species. For transthyretin, low molecular weight oligomers as well as cold shocked native tetrameric transthyretin have been identified as toxic [1]. This emphasizes the importance of structural characterization of these toxic oligomers, as well as elucidation of the fibrillation mechanism and the potential presence of toxic on-pathway oligomers.

The present study focuses on structural characterization of the early transthyretin oligomers. Two approaches are taken: One focusing on a structural characterization and comparison of the cold shocked native tetramer and the non-toxic tetramer formed at room temperature using various techniques. Small Angle X-ray Scattering (SAXS) revealed some structural differences between the two tetramers as a function of transthyretin concentration. Interestingly, it is the cytotoxic cold shocked native tetramer which most resembles the crystal structure of tetrameric transthyretin. Another approach focuses on examining whether significant amounts of oligomers are present during the fibrillation process, as previously has been shown for insulin fibrillation using SAXS [2].

References:
1. Sörgjerd K et al (2008) Biochem. Biophys. Res. Commun., 377 (4) 1072.
2. Vestergaard B, Groenning M et al (2007) PLoS Biology, 5, e135.

Poster No. I-12

LIPID ACTIVATORS AND INHIBITORS OF APOLIPOPROTEIN AMYLOID FIBRIL FORMATION

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The plasma apolipoprotein family is derived from a common ancestor and is characterized by the presence of several class A amphipathic helical regions postulated to mediate lipid binding. The apolipoprotein family has an unusual propensity to form amyloid fibrils *in vivo*. In addition to the amyloid-like properties of apoB in LDL, other members that form amyloid fibrils include apoA-I, apoA-II, apoA-IV, apoC-II, apoE, and the apolipoprotein–related proteins, α–synuclein and SAA. This high representation suggests that the low conformational stability of apolipoproteins in the absence of lipids underlies their high propensity to form amyloid fibrils.

We have developed human apoC-II as a representative plasma apolipoprotein that readily forms amyloid fibrils in a lipid-dependent manner. A significant feature of the activation of apoC-II fibril formation in the presence of sub-micellar phospholipids is the rapid appearance of a tetramer followed by a slow isomerisation that precedes fibril formation. To explore the specificity for the action of lipids on fibril formation, we screened a total of 95 lipids and detergent-like compounds at submicellar concentrations. Several activators and inhibitors were identified based on their effect on ThT fluorescence development by apoC-II. Our hypothesis, arising from these initial studies, is that activator lipids promote nucleation and readily dissociate during fibril growth, while lipid inhibitors promote oligomers but bind more strongly and prevent elongation into mature fibrils. These studies reveal the essential structural characters of lipid activators and inhibitors and their specificities for apolipoprotein amyloid fibril formation.

Poster No. I-13

SERADAN: A MODEL PEPTIDE FOR PATHOGENIC FIBRILLATION

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Familial Danish Dementia (FDD), a rare familial neurodegenerative disease, caused by a mutation in the BRI gene leading to accumulation and deposition of amyloid Danish (ADan), a 34 amino acid peptide [1]. The disease is fatal with death occurring at the age of 50-60 [2]. In previous studies it has been shown that ADan interacts with lipid vesicles and that ADan aggregates into fibrillar structures in the presence of lipid [3]. It has further been shown that the modified peptide SerADan display some but not all of the classic ß-sheet fibril structures [4]. The aim of the present study is to analyze the structures formed during fibrillation to further elucidate the fibrillation mechanism and modifiers of this. In the present study it has already been found that the preparation of uniformly monomeric species prior to the fibrillation is of utmost important for the fibrillation process. The success of the monomerisation step is further believed to be a sign of the presence of impurities.

References:

1. Kim, S.H., Creemers, J.W., Chu, S., Thinakaran, G., Sisodia, S.S. 2002. Proteolytic processing of of familial British dementia-associated BRI variants: evidence for enhanced intracellular accumulation of amylogenic peptides. *J. Biol. Chem. 277:1872*-1877.
2. Vidal, R., Revesz, T., Rostagno, A., Kim, E., Holton, J.L., Bek, M., Bojsen-Moller, M., Braendgaard, H., Plant, G., Ghiso, J., Frangione, B. 2000. A decamer duplication in the 3' region of the BRI gene originates an amyloid peptide that is associated with dementia in a Danish kindred. *Proc. Natl. Acad. Sci. U.S.A. 97:4920-4925*.
3. Nesgaard, L., Vad, B., Christensen, G., Otzen, D.E. 2009. Kinetic partitioning between aggregation and vesicle permeabilization by modified ADan. *Biochimica et Biophysica Acta. 1794:84-93*.

4. Petersen, S.V., Jeppesen, M.D., Bjerring, M., Hein, K.L., Christensen, G., Enghild, J.J., Otzen, D.E., Kristensen, T., Nielsen, N.C. Preparation of SerADan fibrils for solid-state NMR structural analysis: Expression, purification, and fibril formation. Not yet published.

Poster No. I-14

β_2-MICROGLOBULIN TRANSIENTLY POPULATES A LONG-LIVED UNFOLDED STATE

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β_2-microglobulin (β_2m) is a highly soluble globular protein. In patients with hemodialysis-related amyloidosis (DRA), however, the protein becomes amyloidogenic, precipitates in joints and connective tissues, and causes impaired function. β_2m has been extensively investigated by X-ray crystallography and NMR in the attempt to uncover the underlying cause for its amyloidogenic propensity [1]. These studies suggest that β_2m form aggregates directly from a native-like folded conformation. We have recently discovered, however, that β_2m undergoes a transient global cooperative unfolding with a long-lived unfolded state at physiological conditions [2]. We have also shown that a correlation exists between the unfolding kinetics and the propensity for fibrillation which strongly suggests that this transient unfolding is the first step in the molecular mechanism for the amyloid formation [2]. We have now investigated β_2m at a range of acidic pH-values to determine the transient unfolding kinetics at conditions where β_2m wt is known to fibrillate in vitro. Amide hydrogen ($^1H/^2H$) exchange monitored by mass spectrometry was used to measure the unfolding kinetics. The minimum lifetime of the unfolded state could be inferred from the intrinsic amide hydrogen exchange rates of the residues that undergo exchange upon cooperative unfolding. This yields a minimum lifetime of ~3 min at acidic pH. With such a long-lived unfolded state, the probability of bimolecular encounters between two unfolded molecules becomes very high. Such a bimolecular complex is a likely nucleation point for further aggregation and fibrillation.

References:

1. Chiti, F.; Dobson, C. M. *Nat. Chem. Biol.* **2009**, *5*, 15-22 and references herein.
2. (a) Heegaard, N. H. H.; Jørgensen, T. J. D.; Rozlosnik, N.; Corlin, D. B.; Pedersen, J. S.; Tempesta, A. G.; Roepstorff, P.; Bauer, R.; Nissen, M. H. *Biochemistry* **2005**, *44*, 4397-4407 (b) Jørgensen, T. J. D.; Cheng, L.; Heegaard, N. H. H. *Int. J. Mass Spectrom.* **2007**, *268*, 207-216.

Poster No. I-15

PROBING THE FIBRIL TOPOLOGY OF α–SYNUCLEIN BY SOLID-STATE NMR SPECTROSCOPY

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The pathological hallmark of Parkinson's disease (PD) is the deposition of intracytoplasmic neuronal inclusions termed Lewy bodies. The major components of Lewy bodies are amyloid fibrils of the protein, α–synuclein (AS), a natively unfolded 140 amino acid long cytoplasmic protein. Upon aggregation, a large fraction of α–synuclein undergoes a transition from random coil to the cross β-structure typically seen in the amyloid fibrils. Various techniques including solid-state NMR have been

used to prove that the AS can have at least two distinct morphologies. Both these forms have similar secondary structures in the rigid core region. We initiated a detailed structural investigation of the straight-type of fibril using solid-state NMR. In this contribution, we will present various 2D solid-state NMR MAS experiments that enabled us to sequentially assign most of the residue coming from the fibril core. A combination of through-space ssNMR transfer methods and water-edited experiments provided a topological arrangement of the β-strands in the core of α–synuclein fibril. Further, we used a ^{13}C, ^{15}N mixed labelled sample to investigate the arrangement of protofilament in the fibril core. Our results indicate that the b-strands of the protofilaments are arranged in an in-register parallel fashion.

Poster No. I-16

SMALL ANGLE X-RAY SCATTERING OF GNNQQNY FIBRILS

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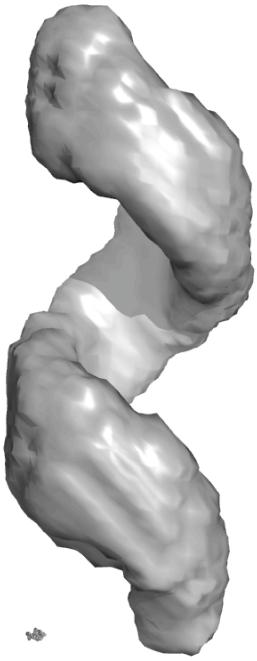
Inspired by the results obtained using small-angle X-ray scattering (SAXS) on the insulin nucleus and fibril [1] and other similar experiments (unpublished), work has been initiated on obtaining structural information of the species in the GNNQQNY fibrillation process using SAXS.
This peptide was chosen as the crystal structure is known [2,3]. By combining the high resolution information on the packing in the crystal structures with other available information from ssNMR [4], fibre diffraction, TEM etc., the aim is for the SAXS data to bridge all the information available to achieve a quasi-high resolution structure of the fibril. A model of the mature fibril will be presented along with preliminary results from further scattering experiments carried out during the fibrillation process.



Average Aβ initio structure of the GNNQQNY fibrils. A single peptide (pdbid 1yjp) is shown in the bottom left for comparison.

References:
1. Vestergaard B, Groenning M, Roessle M, Kastrup JS, van de Weert M, Flink JM, Frokjaer S, Gajhede M, Svergun DI (2007). *PLoS Biol.*, 5, e134
2. Nelson R, Sawaya MR, Balbirnie M, Madsen AØ, Riekel C, Grothe R, Eisenberg D (2005) *Nature*, 435, 773-778
3. Sawaya MR, Sambashivan S, Nelson R, Ivanova MI, Sievers SA, Apostol MI, Thompson MJ, Balbirnie M, Wiltzius JJ, McFarlane HT, Madsen AØ, Riekel C, Eisenberg D (2007) *Nature*, 447, 453-457
4. van der Wel PCA, Lewandowski JR, Griffin RG (2007) *JACS*, 129, 5117-5130

Poster No. I-17

AGGREGATION OF THE NEUROBLASTOMA-ASSOCIATED MUTANT (S120G) OF THE HUMAN NUCLEOSIDE DIPHOSPHATE KINASE-A/NM23-H1 INTO AMYLOID FIBRILS

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The human nucleoside diphosphate (NDP) kinase, product of the nm23-H1 gene, is a metastasis suppressor protein in addition to its classical function in the nucleotide metabolism. A mutant S120G identified in neuroblastomas, posses native three-dimensional structure and enzymatic activity but displays reduced conformational stability. Moreover, the urea denatured protein is unable to recover its native state upon dilution. It accumulates as a folding intermediate with the characteristics of a molten globule state, as deduce from biophysical studies. We report here the effect of temperature, protein phosphorylation and chemical chaperones on the equilibrium between the native monomer and ”molten globule” folding intermediate states. Comparison of the human NDP kinase A with other hexameric NDP kinases showed that the S120G mutation increased the tendency to populat the folding intermediate state, already present in the wild-type protein.
The folding intermediates are known to be involved in amyloidogenesis. It was not a surprise to discover that the S120G mutant of the NDP kinase A aggregated into amyloid fibrils. The microscopic, spectroscopic and tinctorial properties confirmed the amyloid character.

Poster No. I-18

INVESTIGATING THE ENERGY LANDSCAPE OF S6 CROSS FIBRILLATION

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In this study we have implemented a cross fibrillation strategy, feeding different S6 fibril species (wild type and mutants) with different S6 monomer species. This heterogeneous fibrillation is a systematic approach to correlate monomer properties [1] with the ability to get incorporated in different fibril species. Elongation kinetics is followed with ThT fluorescence and the polymorphism of the ensuing fibrils is analyzed with different techniques including Fourier-transform Infrared and Atomic Force Microscopy. Kinetic measurements on $S6^{wt}$ seeds elongated with different S6 mutants show a fibril growth rate that is inversely proportional with the nucleation lagtime of the monomeric species when fibrillated homogeneously (correlation coefficient r=0.76) [2], as proposed by Marcus Fändrich [3]. Parallel with kinetic measurements, we apply a second dimension investigating the thermodynamics of the elongation phase in cross fibrillation utilizing Isothermal Titration Calorimetry, analog to a former study by Kardos and coworkers [4]. We expect that the kinetic and thermodynamic data will reveal an interesting picture of the energy landscape of S6 cross fibrillation e.g. species barrier, fibrillation nucleus [2] and elongation model.

References:

1. Otzen, D.E. and M. Oliveberg, *Conformational Plasticity in Folding of the Split β-α-β Protein S6: Evidence for Burst-phase Disruption of the Native State.* Journal of Molecular Biology, 2002: p. 613-627.
2. Pedersen, J.S., G. Christensen, and D.E. Otzen, *Modulation of S6 Fibrillation by Unfolding Rates and Gatekeeper Residues.* Journal of Molecular Biology, 2004. **341**: p. 575-588.
3. Fändrich, M., *Absolute Correlation between Lag Time and Growth Rate in the Spontaneous Formation of Several Amyloid-like Aggregates and Fibrils.* Journal of Molecular Biology, 2007. **365**: p. 1266-1270.
4. Kardos, J., et al., Direct Measurement of the thermodynamic Parameters of Amyloid Formation by Isothermal Titration Calorimetry. Journal of Biological Chemistry, 2004. **279**: p. 55308-55314.

Poster No. I-19

MECHANICAL PERTURBATION AND PROTEIN AGGREGATION

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Shaking or stirring accelerates protein aggregation. The effect poses a major challenge to production facilities in the pharmaceutical and food industries, whereas in biochemical research it is widely used to speed up aggregation assays, often leading to the formation of amyloid which is intimately involved in many neurodegenerative diseases such as Parkinson's and Alzheimer's.
The project's aim is to develop unifying concepts for the understanding of protein aggregation under mechanical stress. The ambition is both to describe general trends, by observing a range of different proteins subjected to several different types of mechanical stress, and to develop and evaluate models for the aggregation process, by detailed analysis of a few selected cases. The models include the peptide hormone glucagon, known for its polymorphism [1], and insulin. The experimental techniques used for the characterization include ThT binding followed by fluorescence, linear dichroism [2] and microscopy.

References:

1. Pedersen J. S., Dikov, D., Flink, J. L., Hjuler, H. A., Christiansen, G., and Otzen, D. E. (2006) J. Mol. Biol. 355, 501-523.
2. Adachi R., Yamaguchi K., Yagi H., Sakurai K. Naiki H. and Goto Y. (2007) J. Biol. Chem. 282, 8978-8983.
3. Akkermans C., van der Goot A. J., Venema P., Van der Linden E., Boom R. M. (2007) Food Hydrocolloids 22, 1315-1325

Poster No. I-20

INSIGHTS INTO AMYLOID STRUCTURE FROM SHORT ASSEMBLING PEPTIDES

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The spontaneous self-association and aggregation of polypeptides into higher order structures, known as amyloid, is associated with a number of neurodegenerative diseases. Amyloid is characterised structurally by cross-beta sheet architecture. However atomic resolution structures of amyloid have remained largely elusive due to the inherent insolubility and polymorphism in amyloid fibres. In an effort to ascertain greater structural detail, of the amyloid fibre, model systems of short polypeptides have been established. These model peptides, sometimes identified by amyloid propensity algorithms, designed completely from scratch or taken from full-length amyloidogenic sequences, are intriguing in that they retain their ability form amyloid fibres. It is logical that shorter sequences will have fewer possible structural conformations. Furthermore discrepancy between sequences in these models reveals important information about how sequence identity drives amyloid formation making these ideal candidates for the study of amyloid structure and formation.
Here we present our findings on the assembly and final structure of several short amyloidogenic peptides characterised using X-ray fibre diffraction, transmission electron microscopy and circular dichroism.

Poster No. I-21

NOVEL INSIGHTS INTO INSULIN INJECTION AMYLOIDOSIS

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Neurodegenerative diseases and systemic amyloidosis share a common molecular mechanism: conformational changes of soluble forms of peptides and proteins which aggregate into amyloid fibrils. Insulin Injection Amyloidosis is a peculiar syndrome that occurs in insulin dependent diabetes (1,2). This type of amyloidosis is characterized by insulin fibril formation and deposition subcutaneously. The mechanisms underlying insulin amyloid formation in diabetes patients remains unclear. In the present work, the methylglyoxal effects on the structure, stability and fibrillation properties of human insulin were investigated. The modified insulin lose conformational stability and increases its susceptibility toward aggregation and amyloid fibril formation when compared with non-modified insulin. Here is proposed a novel mechanism for Insulin Injection Amyloidosis, where the high concentration of glycation agents in diabetes patients may play a significant role for insulin amyloid fibril formation and deposition.

References:

1. Dische, F. E., Wernstedt, C., Westermark, G. T., Westermark, P., Pepys, M. B., Rennie, J. A., Gilbey, S. G., and Watkins, P. J. (1988) *Diabetologia* **31**, 158-161
2. Swift, B. (2002) *Diabet Med* **19**, 881-882

Poster No. I-22

PROBING DYNAMICS WITHIN AMYLOID FIBRILS

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A host of diseases involve deposition of proteinaceous amyloid fibrils which are highly-ordered, non-covalent polymers that contain cross-β architecture. Despite great interest in these fibres, knowledge of the atomic structure of amyloid is limited due to the difficulty of studying these large heterogeneous biomolecules with any single biophysical method. We have studied the manner in which the polypeptide chain of β_2-microglobulin (β_2m), a 99-residue protein that forms amyloid-like fibrils *in vitro* and *in vivo*, is accommodated within its fibril architecture. By employing a novel method that decouples the interfering contributions of dynamic exchange between fibrillar and soluble material in NMR structural analyses, we discern which regions of β_2m are protected in the core of the fibrils, which are exposed, and which are dynamic.

Poster No. I-23

PROTEIN SEQUENCES ENCODE SAFEGUARDS AGAINST AGGREGATION

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Functional requirements shaped proteins into globular structures. Under these structural constraints, which require both regular secondary structure and a hydrophobic core, protein aggregation is an unavoidable corollary to protein

structure. However, as aggregation results in reduced fitness, natural selection will tend to eliminate strongly aggregating sequences.
The analysis of distribution and variation of aggregation patterns in the human proteome using the TANGO algorithm confirms the findings of a previous study on several proteomes: the flanks of aggregation-prone regions are enriched with charged residues and proline, the so-called gatekeeper-residues. Moreover, in this study we observed a widespread redundancy in gatekeeper usage. Interestingly, aggregating regions from key proteins such as p53 or huntingtin are among the most extensively gatekeeped sequences. As a consequence, mutations that remove gatekeepers could therefore result in a strong increase in disease-susceptibility. In a set of disease-associated mutations from the UniProt database, we find a strong enrichment of mutations that disrupt gatekeeper motifs. Closer inspection of a number of case studies indicates clearly that removing gatekeepers may play a determining role in widely varying disorders such as van der Woude syndrome, X-linked Fabry disease and limb-girdle muscular dystrophy.

Poster No. I-24

AGGREGATION AND FIBRILLAR PROPERTIES OF MONELLIN MUTANTS

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The role of intra-protein interactions in protecting a protein form fibrillation was investigated in a series of monellin mutants. The sweet-tasting protein monellin is naturally composed of two chains, MNA and MNB, and has a tendency to aggregate and form amyloid fibrills in certain conditions. Recombinant single chain monellin, scMN, with MNA covalently joined after MNB to form a protein composed of subdomains B and A, has retained sweetness, increased stability, decreased aggregation propensity and decreased amyloid formation tendency. Here we have produced and investigated the parent scMN and a series of seven scMN variants. All seven mutants were found in inclusion bodies, and purified after solubilization in buffer with urea and salt. The thermal stability of the proteins was studied by circular dichroism spectroscopy. The time dependence of the formation of amyloid fibrils was studied by monitoring the temporal development of thioflavin T fluorescence. We observed reduced thermal stability and increased aggregation tendency of the variants, which is concentration dependent and may be induced by heat treatment and/or by incubation of the protein solution over time. A correlation is observed between the apparent midpoint of thermal denaturation and the lag phase for fibrillation. Our findings are discussed in terms of the 3D structure of monellin and the perturbation of the contact surface between subdomains A and B.

Poster No. I-25

STRUCTURAL DISTORTION AND TRANSIENT OLIGOMERIZATION IN THE MISFOLDING OF CU/ZN SUPEROXIDE DISMUTASE

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The neurodegenerative disorder amyotrophic lateral sclerosis (ALS) is linked to aggregation of Cu/Zn superoxide dismutase (SOD1). More that 100 different familial mutations in SOD1 that increase the risk for ALS have been identified. The molecular events that trigger the misfolding of SOD1 and how SOD1 gains the toxic function associated with ALS however remain unknown.
Here we have used nuclear spin relaxation measurement and site-directed spin labeling to characterize transient structural distortions in SOD1. In metal-free SOD1 the major folded state is in equilibrium with a minor (< 1%) excited state. In the excited state, which structurally is off the unfolding path, the edges of the β-sheets are perturbed resulting in exposure of aggregation potent strands. Metal-free SOD1 furthermore form transient oligomers. Comparing the structural perturbations in the minor state of wild-type SOD1 with the perturbations in the minor state of ALS-linked mutants shows that although the differences are subtle the ALS-linked mutants have a significantly higher population of the minor state. Furthermore, the structures of the minor states of the mutants are more perturbed than the wild-type.
The results suggest a unified mechanism to explain both the probable role of wild-type SOD1 in sporadic ALS and the increased aggregation propensity of ALS-linked familial mutations in SOD1.

Poster No. I-26

EFFECT OF FIBRIL FRAGMENTATION ON THE PHYSICAL AND BIOLOGICAL PROPERTIES OF AMYLOID ASSEMBLY

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Many neuro-degenerative and age-related diseases are associated with the formation of protein aggregates known as amyloid. Despite an increasing number of proteins and peptides being recognised as amyloidogenic, the molecular mechanisms of amyloid formation remain elusive. In particular, the distribution of species formed during different stages of assembly, the structural properties of populated oligomeric species and the nature of the fibril product itself remain unclear. Because the understanding of how amyloid self-assembly occurs is of paramount importance for a molecular interpretation of amyloidosis and for the rational development of therapies against amyloid disease, we have recently characterised in detail the assembly mechanism of beta-2-microglobulin long-straight fibrils [1], where fragmentation was revealed as the dominating secondary process accelerating fibril assembly. Thus, fibril fragmentation is a key secondary process that may prove to be significant in hastening the onset of amyloid diseases *in vivo*. Here, we report the detailed quantitative characterisation of the effects of agitation promoted fragmentation on the physical and biological properties of beta-2-microglobulin fibrils. Using tapping mode atomic force microscopy (TM-AFM) and statistical single particle image analysis we show that agitation does not perturb the molecular architecture of the fibrils, despite decreasing their length distribution significantly. Detailed statistical analysis yielded a quantitative correlation between the extent of fibril fragmentation and the seeding efficiency, precisely as predicted by the nucleated assembly model of long-straight fibrils with fragmentation secondary process. The data are discussed in the context of the effect of fragmentation on fibril load and the consequences for the development of amyloid disease.

References:

1. Xue, W.-F., Homans, S.W., Radford, S.E. (2008) Systematic analysis of nucleation-dependent polymerisation reveals new insights into the mechanism of amyloid self-assembly. *Proc. Natl. Acad. Sciences. USA.* **105**, 8926– 8931.

BENZON SYMPOSIUM No. 56

FUNCTIONAL AND PATHOGENIC PROTEIN AGGREGATION

AUGUST 24 – 27, 2009, COPENHAGEN, DENMARK

Organizing committee:

Daniel Otzen (Aarhus), Poul Henning Jensen (Aarhus), Sven Frøkjær (Copenhagen) & Niels Borregaard (Copenhagen)

Abstracts - TUESDAY, August 25, 2009

MEMBRANE PENETRATION AND PRION-LIKE PROPAGATION OF POLYGLUTAMINE AMYLOIDS

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Sequence-specific nucleated protein aggregation is closely linked to the pathogenesis of most neurodegenerative diseases and constitutes the molecular basis of prion formation. Here we report that fibrillar polyglutamine peptide aggregates can be internalized by mammalian cells in culture where they gain access to the cytosolic compartment and become co-sequestered in aggresomes together with components of the ubiquitin-proteasome system and cytoplasmic chaperones. Remarkably, these internalized fibrillar aggregates are able to selectively recruit soluble cytoplasmic proteins with which they share homologous, but not heterologous amyloidogenic sequences and to confer a heritable phenotype upon cells expressing the homologous amyloidogenic protein from a chromosomal locus.

ROLE OF MOLECULAR CHAPERONES IN NEURODEGENERATIVE MISFOLDING DISEASE

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The folding status of the cellular proteome is under constant surveillance by the molecular chaperone network which functions generally in preventing protein misfolding and aggregation. Chaperones, together with multiple cofactors, have a critical role in mediating folding of newly-synthesized proteins and in conformational maintenance of preexistent proteins. Irreversibly misfolded proteins are targeted for degradation with the aid of chaperones. Assistance of de novo folding is provided by several types of chaperone, often in an ATP-dependent mechanism. In the cytosol, nascent chain-binding chaperones, such as Hsp70, stabilize elongating chains on ribosomes in a non-aggregated state. Folding is then achieved either on controlled chain release from these factors or following polypeptide transfer to downstream chaperones, such as the chaperonin TRiC/CCT, which provides a compartment for single protein molecules to fold in isolation. The cytosolic chaperone machinery also has an important role in controlling protein misfolding and aggregation in the context

of neurodegenerative disorders, such as Parkinson's and Huntington's disease (HD). Specifically, Hsp70 and chaperonins can cooperate to prevent the formation of toxic protein oligomers. A reduction in the capacity of the chaperone system during aging may be critical in the manifestation of these late-onset diseases, suggesting up-regulation of chaperones as a possible therapeutic strategy.

THE STRESS OF MISFOLDED PROTEINS IN NEURODEGENERATIVE DISEASES AND AGING

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The health of the proteome depends upon protein quality control and the ability of the proteostasis network to regulate the proper synthesis, folding, translocation, and clearance of proteins. This network is challenged constantly by environmental and physiological stress, ageing, and the chronic expression of misfolded proteins as occurs in neurodegeneration and other diseases of protein conformation. The expression of Huntingtin polyQ expansion proteins or mutant SOD1 in *C. elegans* results in aggregation toxicity and interferes further with the folding and stability of other metastable proteins, which, in turn, further disrupts diverse signaling and regulatory pathways leading to cellular dysfunction and organismal failure. In part this is due to the collapse of proteostasis that occurs in multiple tissues early in adult ageing. Proteostasis can be restored by enhancing the protective stress response activators Hsf1 and Daf16 that are also essential for lifespan. Hsf1 is regulated post-translationally by the NAD-dependent sirtuin, SIRT1, that maintains Hsf1 in an active DNA-bound state to ensure the functionality of stress responses and proper expression of chaperones during stress and ageing. At the organismal level, the heat shock response In *C. elegans* is regulated by cell non-autonomous control by the AFD thermosensory neurons that sense temperature and regulate the expression of heat shock genes in other somatic tissues. Collectively, these results reveal that the transmission of the heat shock signal requires active neuronal activity, which serves to integrate temperature-dependent behavioral, metabolic, and stress-related responses that have a direct effect on lifespan.

PROTEOSTASIS

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The chemical information within the polypeptide chain, co- and post-translational modifications of the amino acids comprising the protein, including N-linked glycosylation, and the interactions of the polypeptide with proteostasis network components determine whether a given member of the proteome will fold and function, be degraded, remain natively unfolded or aggregate and create additional proteostatic challenges for the organism. The outset of the seminar will focus on the intrinsic forces that predispose polypeptides to fold, including conformational propensities, hydrogen bonding, the hydrophobic effect as well as the influence of post-translational modifications. The second part of the talk will focus on the extrinsic forces that assist and enable proteome maintenance, and the means by which the proteostasis network enhances protein structure acquisition, function and clearance to facilitate life and avoid loss- and gain-of-function diseases. The influence of the proteostasis network, comprising transcriptional and translational control of protein

synthesis, chaperone- and enzyme-assisted folding, disaggregation activities and degradation activities will be covered. Furthermore, the influence of aging-associated signaling pathways on proteome maintenance will be discussed. The lecture will close with a summary of what we have learned about degenerative diseases associated with protein aggregation and loss-of-function diseases associated with excessive mutant protein misfolding and degradation. Specifically, we will focus on how we are ameliorating these diseases with Proteostasis Regulators, small molecules that readapt the innate biology of proteostasis through signaling pathways that control the Proteostasis Network. Synergistic rescue of protein homeostasis through the use of Pharmacologic Chaperones and Proteostasis Regulators and the demonstration that one Proteostasis Regulator can be used to intervene in multiple protein misfolding diseases will be demonstrated.

References:

1. Balch, W.E.; Morimoto, R.I.; Dillin, A.; Kelly, J.W. "Adapting Proteostasis For Disease Intervention" *Science* **2008** 319, 916-919
2. Mu, T-W.; Ong, D.S.T.; Wang, Y-J; Balch, W. E.; Yates, J.R.; Segatori, L.; Kelly, J.W. ."Chemical and Biological Approaches Synergize to Ameliorate Protein-Folding Diseases" *Cell* **2008** 134, 769-781.
3. Mu, T-W.; Fowler, D. M.; Kelly, J. W. "Partial Restoration of Mutant Enzyme Homeostasis in Three Distinct Lysosomal Storage Disease Cell Lines by Altering Calcium Homeostasis" *PloS Biol.* **2008** 6, 253-265, *e26*
4. Wiseman, R.L.; Powers, E.T.; Buxbaum, J.N.; Kelly, J.W.; Balch, W.E. "An Adaptable Standard For Protein Export from the Endoplasmic Reticulum" *Cell* **2007** 131, 809-821.

ROLE OF THE HEAT SHOCK RESPONSE IN PROTEIN MISFOLDING DISORDERS OF EXTRACELLULAR DEPOSITION

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Familial amyloidotic polyneuropathy (FAP) is a neurodegenerative disease affecting specially the peripheral nervous system. The cause of this life threatening pathology is the extracellular deposition of mutant transthyretin as early non-fibrillar deposits and amyloid in sites that do not synthesize TTR. The mouse models characterized so far do not recapitulate the whole features, since the peripheral nervous tissue is spared. We have generated a new mouse model expressing the human transthyretin V30M in a heat shock transcription factor 1 null background which presents extensive and earlier non-fibrillar transthyretin deposition in distinct organs including the peripheral and autonomic nervous system; previous transgenic models failed to achieve TTR deposition in the nervous system; furthermore, inflammatory and oxidative stress, was observed as in human patients. The results show that heat shock transcription factor 1 regulated genes are involved in FAP. The novel mouse model is of the utmost importance in testing new therapeutic strategies and to explore pathogenic mechanisms associated with FAP and other protein misfolding diseases. We found activation of heat shock responsive genes, namely of hsp 70 and hsp 27 in tissues of FAP patients as compared to controls, which further corroborates the notion and importance of the heat shock response in FAP.

TAUOPATHY MODELS: FROM CELLS TO ANIMALS

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Pathologic tau modifications and filamentous tau inclusions are a histopathologic hallmark of Alzheimer's disease (AD) and other tauopathies. Pathogenic mutations in

the tau gene have been found in the hereditary tauopathy frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) emphasizing that tau-related pathology can be sufficient to cause dementia and indicating a central role of tau pathology also in AD. Cell and animal models are indispensible for studying the role of tau in AD and for testing possible therapeutics. We have established an *ex vivo* model using organotypic hippocampal slice cultures combined with virus-mediated expression of EGFP-tagged tau constructs. Biochemical fractionation, confocal high-resolution imaging and live imaging was employed to determine tau aggregation, effects on neuronal morphology and neurodegeneration. To analyze tau modification and its role in a non-vertebrate animal model, we produced transgenic *Caenorhabditis elegans* strains with a panneuronal expression of human tau and a pseudohyperphosphorylated (PHP) tau construct that mimics AD-relevant tau modification. In addition, a novel transgenic mouse model, expressing PHP tau in forebrain neurons was established. Data from the *ex vivo* model provide evidence for a neurotoxic "gain of function" of soluble tau during AD as a result of structural changes that are induced by tau phosphorylation. In *C. elegans*, disease-like modified human tau induces developmental defects. In transgenic mice, moderate levels of modified tau alone are not sufficient to induce tau aggregation or neurodegeneration. The presentation will focus on how it becomes possible with a combination of cell and animal models to determine and untangle the effects of hyperphosphorylation and aggregation of tau during tauopathies.

SDS-STABLE DIMERS OF THE AMYLOID β-PROTEIN (Aβ) ARE POTENT SYNAPTOTOXINS

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Growing evidence suggests that non-fibrillar forms of Aβ play an important role in Alzheimer's disease (AD). Here we describe the synaptotoxic properties of SDS-stable Aβ dimers present in certain samples of human cerebrospinal fluid (CSF) and extracts of AD brain. Cortical samples of 43 brains were used to prepare extracts and then analyzed for Aβ content using a sensitive IP/Western blot protocol. Analysis of TBS brain extracts revealed the presence of SDS-stable Aβ dimer in 9/14 AD cases with virtually no dimer in samples from cases with other forms of dementia or non-demented controls. Importantly, TBS extracts of human brain that contained SDS-stable Aβ dimers blocked LTP both *in vitro* and *in vivo* and impaired the memory of learned behaviour. Similarly, CSF samples that contained SDS-stable Aβ dimer caused a robust block of LTP, whereas samples that contained only Aβ monomer had no effect. Although these data strongly suggested that Aβ dimers were synaptotoxic, there remained the possibility that co-factors present in CSF and brain were also required. To address this possibility, we tested the effect of pure synthetic disulphide cross-linked Aβ dimers generated using synthetic Aβ1-40 in which serine 26 was substituted with cysteine (Aβ1-40Ser26Cys). The synthetic dimer blocked LTP *in vivo* and *in vitro* with a potency at least 20 times greater than freshly dissolved Aβ monomer. Initial immunological analyses indicate that the synthetic dimer is distinct from monomer and suggest that Aβ dimers may be specifically targeted.

Poster No. II-1

PROTEIN AGGREGATION AS AN INHERENT PART OF AGEING

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The aggregation of specific proteins in a disease setting such as neurodegeneration has been studied extensively. The ageing process plays an important role in disease-related protein aggregation, yet little is known about the extent of "normal" protein aggregation associated with ageing.
Using tandem mass spectrometry and chemical labeling, we compared aggregation-prone proteins extracted from young and middle-aged adult *Caenorhabditis elegans.* We identified proteins prone to aggregate with age in the somatic tissues. Specific categories of proteins were over-represented, implying that not all proteins have the same propensity to aggregate during ageing. To analyze the aggregation process *in vivo*, we chose to express an aggregation-prone candidate, KIN-19, tagged with a fluorescent protein. With age, KIN-19 accumulated as puncta in the pharynx. Importantly, these inclusions did not recover after photobleaching suggesting that these are indeed insoluble accumulations. We showed that the ageing process itself and not solely a change in protein levels, is responsible for KIN-19 aggregation over time. Delaying ageing by reducing insulin/IGF-1 signaling prevented full-blown KIN-19 aggregation. Interestingly, the presence of aggregated KIN-19 aggravated the pathology caused by polyglutamine repeats.
Overall, these results suggest that widespread protein aggregation is an inherent part of ageing and can potentially modify the toxicity of disease-related protein aggregation.

Poster No. II-2

***C. ELEGANS* MODELS FOR FAMILIAL DANISH AND BRITISH DEMENTIAS**

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Familial Danish- and Familial British Dementias (FDD/FBD) are rare neurodegenerative disorders characterized by ataxia and early onset Alzheimer's-like symptoms [1]. Both diseases are caused by mutations near the stop codon of the *BRI2* gene resulting in release of C-terminally elongated amyloidogenic peptides ADan and ABri, instead of the wild-type Bri23 peptide[2]. Wild-type *BRI2* appears to inhibit production and aggregation of the Aβ peptide [3, 4], suggesting that loss of inhibitory function as well as aggregation/toxicity of ADan/ABri could lead to the observed clinical features and early onset Alzheimer's disease (AD).
We have generated transgenic *C. elegans* lines expressing EYFP fusions of ADan/ABri and Bri23 peptides. Animals expressing the ADan peptide exhibit early onset formation of brightly fluorescent aggregates and decreased motility phenotype. In contrast, neither Bri23 nor ABri peptides appears to aggregate or have detrimental effect to health of the animals. The *C. elegans* models represent a new tool that can be used for genetic studies for ADan/ABri related proteotoxicity in vivo. Animals expressing both ADan/ABri/Bri23 and Aβ are being generated to clarify the link between AD and FDD, which could lead to new insights in AD.

References:
1. Rostagno, A., et al., *Chromosome 13 dementias.* Cell Mol Life Sci, 2005. **62**(16): p. 1814-25.
2. Kim, S.H., et al., *Proteolytic processing of familial British dementia-associated BRI variants: evidence for enhanced intracellular accumulation of amyloidogenic peptides.* J Biol Chem, 2002. **277**(3): p. 1872-7.
3. Matsuda, S., et al., *BRI2 inhibits amyloid beta-peptide precursor protein processing by interfering with the docking of secretases to the substrate.* J Neurosci, 2008. **28**(35): p. 8668-76.
4. Kim, J., et al., *BRI2 (ITM2b) inhibits Abeta deposition in vivo.* J Neurosci, 2008. **28**(23): p. 6030-6.

Poster No. II-3

CHARACTERIZATION OF NOVEL GENES FOUND TO ACCELERATE AMYLOID-BETA TOXICITY IN A *CAENORHABDITIS ELEGANS* MODEL FOR ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is the most common type of dementia among elderly. The mechanisms causing AD are still largely unknown, but cerebrovascular deposits of amyloid-beta (Aβ) plaques are one of the hallmarks. However, it is becoming clear that the deposits themselves may not be toxic. On the contrary, these aggregates may provide a protective mechanism whereby the cell immobilizes toxic species of soluble Aβ-oligomers.

The nematode *Caenorhabditis elegans* is an excellent model for studying aging due to its short lifespan and powerful genetics. Since most nematode genes and molecular mechanisms have human homologs, results obtained in *C. elegans* can often be extrapolated to humans. Using the powerful genetic toolbox available in *C. elegans*, several elegant disease models have been established to study neurodegenerative diseases such as AD.

Aβ is generated from cleavage of Aβ precursor protein (APP). To understand APP biology, our collaborators completed a study identifying binding partners of APP binding protein 1 X11a (APPB1) (Bredesen Lab, Buck Institute, unpubl.), 22 of these with good homology to *C. elegans* proteins. In order to test these APPB1 binding proteins in an *in vivo* model, we RNAi inactivated these genes in a *C. elegans* model expressing human Aβ42 in muscle, showing an age-related increase in Aβ-aggregation and paralysis. We have identified at least 3 novel genes that dramatically accelerate toxicity in this Aβ42 worm model. Complementing the RNAi analysis, Western blots probed with anti-Aβ42-ab revealed large differences in the distribution of Aβ oligomers in RNAi treated animals compared to RNAi controls. Interestingly, one of the genes also seems to play a role in the toxicity of protein aggregation in general, as is the case for other Aβ-toxicity modulating genes, e.g. *hsf-1* and *hsp-70*. When we inactivated the gene in a model for Parkinson's disease overexpressing α–synuclein in muscle cells, this lead to an increased formation of vesicles of α–synuclein, as previously reported in this model by others.

Poster No. II-4

LIPIDS REVERT INERT ABETA AMYLOID FIBRILS TO NEUROTOXIC PROTOFIBRILS THAT AFFECT LEARNING IN MICE

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While soluble oligomeric and protofibrillar assemblies of A-amyloid peptide cause synaptotoxicity and potentially contribute to Alzheimer's Disease (AD), the role of mature Abeta fibrils in the amyloid plaques remains controversial. A widely held view in the field suggests that the fibrillisation reaction proceeds "forward" in a near irreversible manner from the monomeric Abeta peptide through toxic protofibrillar intermediates, which subsequently mature into biologically inert amyloid fibrils that are found in plaques. Here we show that natural lipids destabilize and rapidly resolubilize mature Abeta amyloid fibers. Interestingly, the equilibrium is not reversed towards monomeric Abeta but rather towards soluble amyloid protofibrils. We characterized these "backward" Abeta protofibrils generated from mature Abeta fibers and compared them with previously identified "forward" Abeta protofibrils obtained from the aggregation of fresh Abeta monomers. We find that backward protofibrils are biochemically and biophysically very similar to forward protofibrils: they consist of a wide range of molecular masses, are toxic to primary neurons and cause memory impairment and tau phosphorylation in mouse. In addition, they diffuse rapidly through the brain into areas relevant to AD. Our findings imply that amyloid plaques are potentially major sources of soluble toxic Abeta-aggregates which could readily be activated by exposure to biological lipids.

Poster No. II-5

INSIGHTS INTO MECHANISMS OF AMYLOID FORMATION-DISAGGREGATION: INSULIN AMYLOID LABILITY AND INFLAMMATION-RELATED AMYLOIDOSIS IN AGING PROSTATE

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Amyloid formation has recently emerged as a universal phenomenon involved in a wide range of human pathologies and industrial processes based on proteinaceous materials. Significant stability towards environmental conditions and proteases is a new gain-on function of amyloids compared to precursor proteins and a major prerequisite of their undesirable accumulation. In our studies we have focused on the amyloid properties and stability of insulin and disease related pro-inflammatory proteins S100A8/A9.

The amyloid lability of insulin, defined as its propensity either to grow or disaggregate, was assessed under a wide range of pH conditions and also depending on the maturity of the amyloid seeds. Three-dimensional lability landscape in coordinates of pH and amyloid aging demonstrates that the insulin amyloids propensity to grow or dissociate strongly depends on the ionization state of amino acid side chains and also on the packing density of fibrils at different stages of their maturation. It is important to note that even mature fibrils do not possess a protease K resistant core and can be digested with the rate of 2 nm/min as estimated by a real-time AFM.

We have shown that the amyloidosis of inflammatory proteins is directly linked to such major age-related phenomenon as prostate tissue remodeling in middle-aged and elderly men. We have analysed the prostate corpora amylacea inclusions from human patients undergoing radical prostatectomy due to prostate cancer and found that their major component is pertinacious amyloid form of pro-inflammatory proteins S100A8/A9. Pro-inflammatory S100A8 and S100A9 proteins are established biomarkers of numerous inflammatory and cancer conditions. We demonstrated that material closely resembling *ex vivo* corpora amylacea can be produced S100A8/A9 proteins *in vitro* in the presence of Ca or Zn. We have also found DNA and proteins from *E. coli* in *ex vivo* corpora amylacea, suggesting that their formation is also

associated with bacterial infection, accompanied by the activation of macrophages and by an increase in the concentrations of S100A8/A9 in the surrounding tissues. These findings together suggest a close link between bacterial infection, inflammation, and the deposition of the amyloid form of pro-inflammatory proteins in the prostate gland, such that a self perpetuating cycle is triggered that can increase the risk of malignancy in the ageing prostate.

Poster No. II-6

QUANTITATION OF AMYLOID BETA OLIGOMERS IN CSF FROM PATIENTS WITH ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disease defined by the presence of senile plaques and neurofibrillary tangles in the brain. The senile plaques and neurofibrillary tangles, which are pathognomonic for AD, consist of aggregated proteins. The senile plaques mainly contain an abnormally processed amyloid beta peptide (1-42), which is more prone to aggregate than the native amyloid beta (1-40) fragment. In recent years, the toxicity of the amyloid beta-rich senile plaques has been questioned and it has been suggested that the toxic species of amyloid beta in AD is in fact the soluble oligomers of the peptide. We have previously shown that the amyloid beta 1-42 levels in cerebrospinal fluid (CSF) from patients with AD are decreased compared to control subjects. The diagnostic sensitivity of decreased amyloid beta 1-42 is high in AD patients, but the specificity against other neurodegenerative diseases is low.
In this study we develop an ELISA method for the quantification of oligomeric amyloid beta in CSF using in-house developed monoclonal antibodies specific for the N-terminal of the amyloid beta peptides. Levels of oligomeric amyloid beta in CSF are correlated with currently used AD laboratory CSF-biomarkers, i.e., total tau, phospho-tau and amyloid beta 1-42.

Poster No. II-7

RISK FACTOR FOR ALZHEIMER'S DISEASE: PATHOLOGICAL ABETA 40/42 RATIO FACILITATES FORMATION OF STABLE NEUROTOXIC OLIGOMERS

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Alzheimer disease pathology has been related to the toxic effects of the beta-amyloid peptide (Abeta). Abeta is produced by cleavage from Amyloid Precursor Protein (APP) by a combined action of beta- and gamma-secretase. The most abundant beta-amyloid species produced and present in amyloid plaque deposits in the brains of Alzheimer's disease patients are 40 and 42 amino acids long, which comprise ~90% and 10% respectively of the total Abeta pool. Published data suggest that this ratio is often shifted toward a 70%/30% ratio in Alzheimer's disease patients. APP and Presenilin mutations associated with the disease affect the ratio of peptides with

different length. We investigated how different ratios of Abeta peptides influence amyloid aggregation process. We performed a morphological, biophysical and biochemical characterisation of physiological and pathological Abeta (1-40) to Abeta (1-42) ratios during the aggregation reaction. We studied the neurotoxic effects and memory formation in mice injected with those preparations. We found a remarkable and distinct effect of Abeta (1-40) to Abeta (1-42) ratios on the generation of stable neurotoxic oligomers and protofibrils. The differences in effects on neuronal cell death and on memory formation in mice is reflected in differences in the aggregate morphology and aggregation kinetics of the different Abeta preparations. We conclude that the relative ratios of peptides with varying length affect stability and toxicity of the aggregates and that this quality of Abeta is more important than the absolute quantity for the generation of toxic amyloid species.

Poster No. II-8

PROTEOMICS INVESTIGATIONS OF MITOCHONDRIA FROM GENE-MODIFIED MICE EXPRESSING DECREASED LEVELS OF THE MITOCHONDRIAL HSP60 CHAPERONE

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Mutations in the mitochondrial chaperone Hsp60 are associated with two neurological diseases: a dominantly inherited form of spastic paraplegia (SPG13) and a severe autosomal-recessively inherited neurodegenerative disorder linked to brain hypomyelination and leukodystrophy. Our investigations of the effects of disease-causing mutations in Hsp60 have indicated that the mutant Hsp60 protein has partial activity, and exerts no marked dominant negative effect. These studies suggest that the diseases are caused by decreased Hsp60 activity leading to misfolding and/or aggregation of mitochondrial proteins, with severity correlating to the degree of residual Hsp60 function.

We have produced heterozygous Hsp60 knockout mice carrying a genetrap insertion in intron 2 in one allele of the *Hspd1* gene, which encodes the HSP60 protein. Homozygosity for the $Hspd1^{GT}$ allele leads to early embryonal lethality. Heterozygous $Hspd1^{WT/GT}$ mice display approximately 50% level of Hsp60 mRNA and protein in all tissues examined (brain, liver, skeletal muscle and heart) and represent a model for decreased Hsp60 activity.

To study the effects of Hsp60 chaperone deficiency on the mitochondrial proteome and to identify proteins which misfold or aggregate, we have set up a mass spectrometric method. Mitochondria were isolated form brain tissue of heterozygous $Hspd1^{WT/GT}$ mice and controls and quantiative mitochondrial protein profiling was performed after iTRAQ labeling using a high mass accuracy mass spectrometer LC-MS/MS system (nano-LC in line with LTQ Orbitrap™). Our investigations confirmed reduced levels of Hsp60 and identified a number of proteins, which were significantly decreased in their levels and therefore may represent proteins whose folding is affected by decreased Hsp60 chaperone activity. Interestingly, one of the proteins displaying reduced levels was mitochondrial superoxide dismutase. The implications of our results will be discussed.

Poster No. II-9

INVESTIGATION OF THE α–SYNUCLEIN PATHWAY IN THE OLN CELL MODEL

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Some neurodegenerative diseases such as Parkinson's disease and Dementia with Lewy Bodies are characterized by the presence of intraneuronal inclusions composed mainly of α–synuclein protein fibrils. Phosphorylated α–synuclein has been found in these neuropathological lesions, pointing to its crucial role in the pathogenesis of synucleinopathies. Moreover it has been found that the nuclear localization of α–synuclein may play a role in the disease development.

To further investigate the role of α–synclein phosphorylation and its localization in the nucleus, we have made various α–synuclein constructs with nuclear localization signals (NLS) and nuclear export signal (NES), which allow us to direct the protein to the nucleus or the cytoplasm, respectively. Experiments with wt α–synuclein or the phosphorylation-incompetent α–synuclein mutant S129A with these localization signals will be performed in the rat oligodendroglial (OLN) cell model [1], where we are also able to chemically inhibit aggregation and phosphorylation of α–synuclein. With the OLN cell model we will furthermore permit to establish whether p25α plays a role in the translocation of α–synuclein.

The role of phosphorylation and/or translocation of α–synuclein bring new perspective to therapeutic approaches focusing on controlling the toxicity of α–synuclein.

References:

1. Kragh CL, Lund LB, Febbraro F, Hansen HD, Gai WP, Richter-Landsberg C, Jensen PH: alpha - synuclein aggregation and Ser129 phosphorylation dependent cell death in oligodendroglial cells. *J Biol Chem* 2009.

Poster No. II-10

AMYLOID BETA PEPTIDE FIBRIL FORMATION MODULATED BY PHOSPHOLIPID MEMBRANES

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Disease-causing amyloid fibril formation can be modulated by many factors including interactions with biological lipid membranes. An increasing amount of evidence suggests that the process of fibril formation *in vivo* and the mechanism of toxicity both involve membrane interactions. Alzheimer's is probably the most well-known amyloid disease and the amyloid beta peptide is originally cleaved off from the amyloid precursor protein (APP), which is a membrane-incorporated protein. In its C-terminal part, the resulting peptide contains 11 to 14 amino acids originally incorporated into the membrane. This gives the cleaved peptide a significant amphiphilicity, but also electrostatics are believed to be important in the membrane-peptide interaction.

We use recombinant Abeta M1-40 and Abeta M1-42 produced in *Escherichia coli*, which allows us to perform large scale kinetics assays with good statistics where the amyloid formation process is followed in means of thioflavin T fluorescence. The lipid membranes are introduced in the system as large unilamellar vesicles composed of Dioleoyl-phosphatidylcholine (DOPC), Dipalmitoyl-phosphatidylcholine (DPPC) and Sphingomyelin, with and without incorporation of cholesterol. We find that the phase behanviour of the membrane in the vesicles has a large effect on the lag time of the

amyloid formation process for both Abeta M1-40 and M1-42. All membranes increase the lagtime to some degree but DPPC has the largest effect. By comparing different phases we can conclude that the translational diffusion in the membrane seems to be more important than the acyl chain ordering. Negatively charged membranes have also been investigated and no significant difference is detected in lag time when increasing amounts of Dioleoyl-phosphatidylserine (DOPS) are inserted into a DOPC membrane. The kinetics of the amyloid formation itself is highly salt dependent but the membrane induced increase in lag time is observed at both low and physiological salt concentrations.

Poster No. II-11

CHARACTERIZATION OF AMYLOID-BETA DERIVED DIFFUSIBLE LIGANDS (ADDLS) AGGREGATION AND THEIR EFFECTS ON SYNAPTIC TRANSMISSION IN MICE HIPPOCAMPUS SLICE

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The brains of Alzheimer's patients are characterized by accumulation of extra cellular proteins (plaques) which is aggregates of amyloid-beta (Aß) protein. According to the amyloid-cascade hypothesis, Aß is essential for the symptoms and the progression of the disease, but it has not yet been established in which forms of Aß may act as pathogenic agents. Soluble semi-stable oligomeric forms of Aß (Amyloid-Beta Derived Diffusible Ligands, ADDLs) seem to be causally related to the initial memory symptoms of AD. Furthermore, ADDLs interfere with long-term potentiation (LTP) of glutamatergic synaptic transmission which is believed to be closely related to memory formation in the brain.

The aggregation of monomeric Aß into ADDL´s and fibrils is concentration and time dependent and is affected by a large numbers of factors including pH, temperature and various ions in the solvent. In the current project we investigated the aggregation process of solubilized $A\beta_{1-42}$ over time when subjected to normal standardized or more physiological conditions, and tested the effects of varying different divalent cations in the solvent, e.g. Zn^{2+}. Monomerized Aß was incubated for 0, 2, or 7 days in MilliQ-water or physiological solutions. The formation of the Aß - aggregates (ADDLs) was characterized by Western Blot, Atomic Force Microscopy (AFM) and Field Flow Fractionation (FFF). Our data showed that the ADDLs formation process occured slowly over days, initially with formation of smaller oligomers which then later aggregated into oligomers of higher order. Physiological solutions and temperature promoted the aggregation of Aß into higher oligomers and the types of oligomers formed were not identical to the ADDLs formed in MilliQ-water. This suggests that the aggregation process of Aß differ in the two conditions. The different preparations of ADDL's were tested for effects on LTP in vitro on hippocampal slices. LTP induced by theta burst stimulation in hippocampal slices appear to be affected by the addition of various Aß-fractions containing mainly small oligomers.

Poster No. II-12

SYNTHESIS, MEMBRANE INTERACTIONS AND CYTOTOXICITY OF ISLET AMYLOID POLYPEPTIDE AND ITS PRECURSORS

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Human islet amyloid polypeptide (hIAPP) is a 37-residue peptide that is the major component of the amyloid deposits frequently found in pancreatic islets of patients suffering from diabetes mellitus type 2 (DM2). Aggregation of this islet β-cell peptide has been implicated in β-cell failure. Notably, interactions between hIAPP and cellular membranes are considered a cause of hIAPP cytotoxicity and insulin insufficiency. IAPP is synthesized initially as a preprohormone. It has been suggested that in DM2 the processing of this precursor is defective and that aggregation of the processing intermediates prohIAPP and prohIAPP$_{1-48}$ may represent an initial step in formation of islet amyloid. To provide further insight in the interactions between cell membranes and IAPP/precursors, we chemically synthesized human (h) and murine (m) ProIAPP, ProIAPP$_{1-48}$ as well as mature IAPP. mIAPP differs from hIAPP at only 6 residues and cannot form amyloid fibrils. IAPP and its precursors were synthesized by manual Boc-SPPS. ProIAPP and ProIAPP$_{1-48}$ were prepared by SPPS and native chemical ligation. Amyloid fibril formation of IAPP, IAPP-precursors and combinations thereof was studied in solution, as well as in the presence of synthetic membranes and β-cells. Our data revealed that both hIAPP precursors formed amyloid fibrils in solution but not in the presence of membranes. Importantly, in the presence of membranes both precursors were able to inhibit fibrillogenesis of mature hIAPP. Furthermore, while mature hIAPP affected the barrier properties of lipid vesicles, neither of the precursors was able to induce membrane leakage. Our study suggests that the hIAPP precursors do not serve as amyloid initiators, but rather prevent aggregation and membrane damage of mature hIAPP in early stages of its biosynthesis and intracellular transport.

Poster No. II-13

TRANSTHYRETIN V30M FORMS TWO COMPOSITIONALLY DIFFERENT AMYLOID FIBRILS WHICH ARE RELATED TO CLINICOPATHOLOGY IN FAMILIAL AMYLOIDOTIC POLYNEUROPATHY (FAP)

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Familial transthyretin amyloidosis is caused by a mutation in the transthyretin (TTR) gene. More than a hundred mutations have been found and the majority are amyloidogenic. The most common variant is V30M, which is especially found in endemic areas in Sweden, Portugal and Japan. The disease primarily affects the peripheral nerve system and the heart, but manifestations differ between each mutation and interestingly also between individuals carrying the same mutation. The reason for this variation is not known. The only established treatment for the disease today is liver transplantation. This eliminates the mutant TTR in the plasma, as the TTR circulating in plasma is almost exclusively synthesized in the liver. The procedure halts the progression of the disease in most cases, but a rapid continued amyloid deposition of wild-type TTR in the heart has been seen in some individuals.
We have discovered that amyloid fibrils in ATTRV30M patients can have two different types of composition. The fibrils can either contain solely full-length ATTR molecules, or be composed of a mixture of full-length and N-terminally truncated fragments. It seems like the fibril type differs between individuals but not between different organs

of each individual. The two kinds of fibrils are dissimilar in Congo red staining appearance, as amyloid with fragments has a weak and smooth appearance whereas amyloid without fragments is more brilliant and granular. The type of fibrils found in the amyloid of a patient is correlated to disease phenotype. Patients whose amyloid is build of only full-length ATTR have an early onset of disease and only small amounts of cardiac amyloid whereas patients with fragmented ATTR have a late onset and develop restrictive cardiomyopathy due to heavy amyloid infiltration of the heart. We have also found that fibrils with fragments have a greater propensity for wild-type ATTR incorporation than fibrils lacking fragments. This could be an answer to why a progressive deposition occurs post-transplantationally in some patients, but not in others.
In conclusion, TTRV30M forms two types of amyloid fibrils which are intra-individual consistent and related to differences in age at disease onset, clinical manifestations and possibly also to liver transplantation outcome.

Poster No. II-14

COMPARATIVE PROTEOMIC INVESTIGATION OF PARKINSONS DISEASE VARIANTS

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Despite numerous studies examining the etiopathological nature of Parkinsons disease (PD), especially the last ten years, no one has yet found a unifying pathway that ties the various hereditary form of PD to the dominating population of sporadic Parkinson's disease cases. In this study our aim is to examine whether there are significant cellular confounding mechanisms between different pathologic variants of PD seen from a proteomic perspective. To achieve this thorough comparative study we are examining several human brain-type cell line models of PD using the mass spectrometry-based method, stabile isotope labelling with amino acids in cell culture (SILAC). Simultaneous with the proteomic investigation, the cells lines transcription patterns will be characterised by a comprehensive tag-based transcriptomic analysis using our in-house Solexa sequencer and DeepSAGE protocol. To mine the acquired data for information about pathway activation and other types of response related to the PD pathology, the acquired data will be examined using several pathway analysis tools.
At present the quantitative mass spectrometry workflow, including differential centrifugation of lysates into four cell compartmental fractions and further separation by gel-electrophoresis followed by in-gel digest and LC-MS, is implemented and tested through pilot experiments. Shortly, the first complete data set is ready for data analysis.

Poster No. II-15

CELLULAR SECRETION OF A CLEAVED FORM OF P25α DURING α–SYNUCLEIN AGGREGATE CYTOPATHOLOGY

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The brain specific protein p25α accumulates in α–synuclein containing brain inclusions in Parkinson's disease, Lewy body dementia and multiple systems atrophy

and it can stimulate the aggregation of α–synuclein in vitro. Moreover, coexpression of p25α with α–synuclein in oligodendroglial cells initiate a α–synuclein aggregate dependent cellular stress and ensuing cell death. We have been able to demonstrate that cells secrete a C-terminally truncated p25α molecule by the use of a novel antibody. The secretion of cleaved p25α was not altered by changing major cellular proteolytic systems but was enhanced when the cells was subjected to α–synuclein aggregate dependent stress. We hypothesize the cleavage and secretion of p25α represents a cytoprotective mechanism whereby the stressed cells try to clear the cytosol for the stress inducing p25α protein.
The characterization of the p25α cleaving proteinase and its functional role in relation to protection against protein aggregate dependent stress may reveal novel means for reducing aggregate dependent cell stress.

Poster No. II-16

CONCENTRATION DEPENDENCE OF THE FIBRIL FORMATION KINETICS FOR AMYLOID β-PEPTIDE

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Protein aggregation can result in a major disturbance of cellular processes, and is associated several human diseases. The amyloid β peptide (Aβ) seems to play an important role in the pathogenesis of Alzheimer's disease (AD). Aβ is produced from a precursor protein, APP, by specific proteases and is kept at a constant concentration in healthy individuals. The main proteolytic products have 40 and 42 residues, respectively, and the 42 residue peptide is most aggregation prone and of higher significance for disease development. Onset of AD correlates with an imbalance in the ratio of the 42 versus 40 products or increased total concentration. The fibrillar form of Aβ has a characteristic stacking of β strands perpendicular to the long axis of the fiber. The molecular events behind the process leading from native to fibrillar states remain elusive, but accumulated data from many studies suggest that it involves a number of intermediate oligomeric states of different association numbers and structures. Pre-fibrillar oligomers seem to be critical components for development of disease symptoms.
Important questions regard molecular properties of Aβ peptide and its environment which prevent or promote aggregation and amyloid fibril formation. To address these questions we have developed a recombinant expression system with a facile and scalable purification protocol for Aβ(M1-40) and Aβ(M1-42), which relies on inexpensive tools [Walsh et al., 2009]. This allows us to produce large quantities of highly pure monomeric peptide to enable large scale systematic studies. We have also made an effort to eliminate as many sources of experimental error as possible and can now acquire highly reproducible kinetic data on Aβ fibrillation. We will report here the results of large scale systematic studies of the fibrillation kinetics of Aβ and its dependence on peptide concentration, solution composition as well as the influence of foreign surfaces.

Poster No. II-17

α–SYNUCLEIN AGGREGATION AND SER129 PHOSPHORYLATION DEPENDENT CELL DEATH IN OLIGODENDROGLIAL CELLS

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Aggregation of α–synuclein is implicated as a critical factor in several neurodegenerative diseases including multiple system atrophy (MSA), Parkinson's disease (PD), and dementia with lewy bodies (DLB). The affected cell types vary from being primarily oligodendroglial in MSA and neuronal in PD and DLB.
We present a cellular model of oligodendroglial degeneration that shows a fast morphological reorganisation of the microtubules from the cellular processes to the perinuclear region followed by a slowly progressing apoptosis. The process is triggered by coexpression of α–synuclein and the brain specific protein p25/Tppp. P25/Tppp is an oligodendroglial protein that potently stimulates α–synuclein aggregation *in vitro* and which is found in glial cytoplasmic inclusions in MSA and also in Lewy bodies of PD and DLB.
A peptide specific inhibitor of α–synuclein aggregation, non-selective inhibitors of aggregation such as Congo red and baicalein, as well as inhibitors of α–synuclein Ser129 phosphorylation like Casein Kinase 2 and Polo Like kinase has been shown to inhibit degeneration in this model. Thus both Ser-129 phosphorylation and aggregation of α–synuclein are critical parameters
The model is currently being investigated with the aim of using it for a screening of a small molecule library with known mechanism of action in the search of potential targets for therapeutic intervention or early biomarkers.

References:
1. JBC; Lindersson, E; *et al*.; p25α Stimulates α-Synuclein Aggregation and Is Co-localized with Aggregated α-Synuclein in α-synucleinopathies; 280; 7; pp5703-5715 [2005]
2. JBC Paper in press; Kragh, CL *et al*; α-synuclein aggregation and Ser129 phosphorylation dependent cell death in oligodendroglial cells; [2009]

Poster No. II-18

INFLUENCE OF CU(II) ON THE AGGREGATION KINETICS OF ALZHEIMER'S β-AMYLOID

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Using capillary electrophoresis (CE), ThT fluorescence spectroscopy, and atomic force microscopy (AFM), evidence was found that Cu(II) alters the aggregation kinetics and fibrillation of $A\beta_{1-40}$. The effect is dependent on the molar ratio of Aβ:Cu(II) (Fig. 1). Furthermore, Cu(II) causes differences in the morphology of the $A\beta_{1-40}$ aggregates (Fig. 1c); amorphous aggregates and fibrils and were formed with and without Cu(II), respectively.

Conclusions

(1) The spontaneous aggregation kinetics of $A\beta_{1-40}$ fit well to a logistic model (CE and ThT fluorescence)
(2) The aggregation kinetics is markedly changed by the presence of Cu(II) during incubation and the kinetic profiles depend on the molar ratio of Aβ:Cu(II)

(3) Cu(II) changes the morphology of the $A\beta_{1-40}$ aggregates formed from the solutions (AFM)

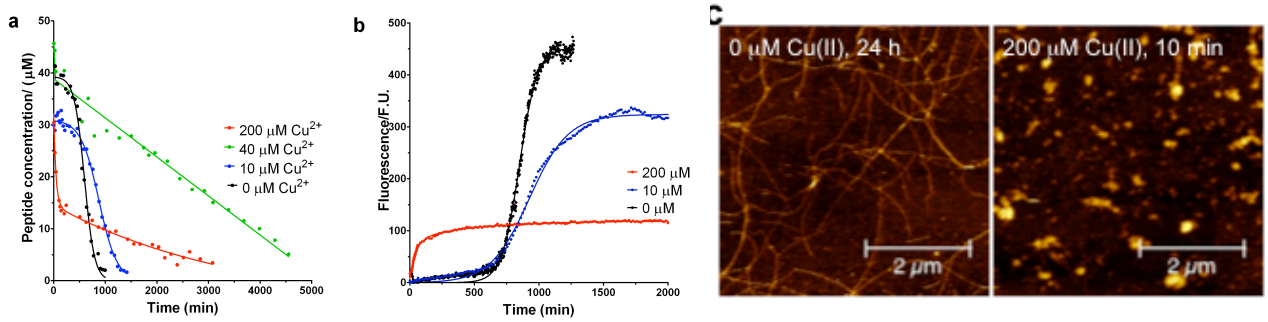

Fig. 1. Aggregation kinetics of $A\beta_{1-40}$ (40 μM) with different Cu(II) concentrations as monitored by a) CE, b) ThT fluorescence c) AFM. Experiments were performed at 37°C, in 20 mM HEPES buffer w. 100 mM NaCl, pH 7.40.

Poster No. II-19

DOMINANT-NEGATIVE INACTIVATION OF WILD TYPE P53 BY A MECHANISM OF MUTANT-INDUCED CO-AGGREGATION

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Missense mutations of the p53 tumour suppressor are the most common genetic change in cancer. Most mutations cluster in the p53 DNA binding domain thereby impairing transcriptional activity that is essential for cell cycle control and apoptosis. Importantly, many of these mutants display dominant-negative behaviour on wild type p53. As native p53 is a tetramer, it was hitherto assumed that the dominant negative effect of mutant p53 results from the formation of mixed wild type/mutant tetramers. Here we show that several important p53 disease mutants form abundant beta-enriched aggregates in various tumour cell lines originating from bone, brain, bladder, pharynx and lymphocyte as well as in tumour tissue from human and mice. Moreover, in SaOS-2 osteosarcoma cells we find that the aggregation propensity of mutant p53 induces wild type co-aggregation resulting in dominant p53 accumulation and inactivation. Dominance is independent from tetramerization but is instead exerted by a short aggregation-nucleating region consisting of a β-strand encompassing residues 251 to 257 in the DNA binding domain. These findings suggest an important role for protein aggregation in the stabilisation of p53 in cancer cell lines and hence for the development of cancer.

Poster No. II-20

NOVEL BIOMARKERS FOR THE DIFFERENTIAL DIAGNOSIS OF ALZHEIMER'S DISEASE AND VASCULAR DEMENTIA

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Alzheimer's disease (AD) is the most common form of dementia found in all human populations worldwide, vascular dementia (VaD) is the second most common form of

dementia. New biomarkers for early and specific diagnosis of AD and VaD are needed to achieve greater insight into changes occurring in the brain and direct therapeutic strategies.
The objective of this study was to develop a multi-marker protein based test for early, differential diagnosis of AD and VaD that improves on the performance of current assays employing single biochemical markers.
Dementia diagnoses and differential diagnoses were made using DSM-IV and ICD-10 criteria. Surface Enhanced Laser Desorption/Ionization (SELDI) TOF-MS was used to differentially profile proteins and peptides in CSF samples from 28 AD patients and 21 patients with VaD.
A total of 19 candidate biomarkers separating AD from VaD were found with ROC values above 0.7. Among them were two truncated forms of Ubiquitin ($p = 0.0001$) as well as Chromogranin B and Secretogranin V fragments ($p = 0.0056$ and $p = 0.0038$, respectively). A multivariate model combining 5 peaks resulted in a ROC value of 0.835
This novel panel of biomarkers could potentially be used to improve early differential disease diagnosis of AD and VaD as well as provide complementary information to help decision making in the development of disease modifying compounds.

Poster No. II-21

ILLUMINATING VARIOUS AMYLOID FIBRILS BY NILE RED

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A variety of diseases are associated with amyloid deposition composed of malfolded protein molecules. Irrespective of the protein sequence and the native protein fold, the formed amyloid fibrils display remarkably similar structures stabilized by cross-beta-sheet core of the individual proteins that are aligned perpendicular to the fiber axis. Also common oligomeric intermediates often appear which display a spherical appearance 5-50 nm in diameter. Assemblies of misfolded proteins are transient and conformationally heterogeneous. Also the likely dynamic interplay between oligomers and fibrils is not at all understood. Hence new methods and molecular probes to investigate these species are of interest to widen our poor understanding of the amyloid diseases.
We have developed methods and molecules to remedy this shortcoming. Data will be presented showing the use of the fluorophore Nile red as a conformational probe for studies of amyloid fibrils of the human prion protein, insulin, lysozyme, transthyretin and A-beta. Nile red fluorescence sensing of amyloid structures is a highly useful complement to the widely used ThT assay. For several amyloid proteins Nile red is insensitive to fluctuations of pH ranging from very acidic (pH 1.6) to neutral (pH 7.5) a trait not demonstrated for ThT. Nile red is sensitive to local polarity rendering it possible to assay surface hydrophobicity of different amyloid fibrils. Further Nile red is fluorescent in the range 600-700 nm making this molecule less sensitive to light scattering and spectral overlap with other molecules. In addition ThT – Nile red FRET is efficient hence co-staining with these amyloidotropic dyes can be used to investigate fibril compactness. As an example we show that amyloid fibrils from A-beta 1-42 grown under different conditions can be differentiated using ThT – Nile red co-staining.

Poster No. II-22

THE IMPACT OF ALZHEIMER'S Aβ OLIGOMERS ON CELLULAR FUNCTION

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β-amyloid, a 39-43 amino acid peptide, is the major component of senile plaques found in the brains of Alzheimer's disease patients. Aβ1-42, has been shown to aggregate with greater propensity compared to its shorter counterpart, Aβ1-40 [1]. Both Alzheimer's peptides are able to assemble to form both toxic oligomers and mature amyloid fibrils. It has previously been shown that accumulation of Aβ1-42 oligomers in the late endosomes/lysosomes results in Aβ1-42 intracellular accumulation, possibly leading to lysosomal leakage and cell toxicity [2].
Using differentiated and undifferentiated human derived neuroblastoma SH-SY5Y cells, treated with various concentrations of soluble oligomeric and insoluble fibrillar Aβ1-42 over a time course, we have assessed type and levels of Aβ1-42 induced cell death. Furthermore, the effect of administering oligomeric and fibrillar Aβ1-42 to the cells in terms of subcellular localisation and degradation has been characterised using immunofluorescence coupled with immunogold labelling and electron microscopy. Complementary electrophysiological studies have been carried out using living snail ganglia to compare and monitor the effect of sub-lethal doses of Aβ oligomers and fibrils and these results show a strong influence on the tonic firing and polarisation of the membrane.

References:
1. D. Burdick, B. Soreghan, M. Kwon, J. Kosmoski, M. Knauer, A. Henschen, J. Yates, C. Cotman, C. Glabe, *J Biol Chem* **1992**, *267*, 546.
2. K. Ditaranto, T. L. Tekirian, A. J. Yang, *Neurobiol Dis* **2001**, *8*, 19.

Poster No. II-23

P25A IS AN AGGREGATE-PRONE PROTEIN THAT IS LINKED TO NEURODEGENERATIVE DISEASES

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Protein aggregation is an underlying feature of most neurodegenerative disorders. It is not yet fully understood how aggregation is related to both protein structure and consequent cytotoxicity. However, some proteins lacking noticable secondary structure, e.g. α–synculein and tau, have been shown to have an intrinsic propensity to form aggregates. These aggregates are commonly found in the brains of patients suffering from Parkinson's or Alzheimer's disease. P25α is a novel protein in the neurodegeneration field. It has been shown to accelerate the aggregation of α–synuclein in vitro and also to co-localize with α–synuclein in Parkinson's disease. Furthermore, p25α has been found in various proteinacious inclusions in other neurodegenerative diseases. Using brain tissue from patients with Alzheimer's disease, Parkinson's disease, Dementia with Lewy bodies and Progressive supranuclear palsy, we show that these diseases are associated with abnormal expression of p25α in neurons in the hippocampus. This expression is manifested by round inclusions as well as a diffuse, cytoplasmic localization of p25α. Using recombinant protein we have found that p25α has a propensity to form aggregates in

the presence of anionic species such as heparin and arachidonic acid. These compounds are also known to stimulate the aggregation of the Alzheimer's disease-associated protein tau. Formation of p25α aggregates is measured using a ThT-assay and secondly verified by sedimentation. Electron- and Atomic Force-Microscopy experiments show that the aggregates have a fibrillar morphology. Further studies reveal that p25α aggregates are insoluble in the detergent Triton X-100, but readily solubilized in SDS. Using tryptic digestion, we show that aggregation of p25α does not markedly change the tryptic cleavage pattern but protects against proteolysis. These result demonstrate that p25α has aggregational properties as well-known disease-associated proteins, and suggests that p25α aggregation may play a role in neurons during disease.

Poster No. II-24

CONCANAVALIN A AGGREGATION AND TOXICITY ON NEUROBLASTOMALAN5 CELL CULTURES

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Concanavalin A (ConA) is a protein belonging to the legume lectins family. It is known to be able to induce Programmed Cell Death in cortical neurons by a mechanism which displays remarkable analogies with the Programmed Cell Death induced by the amyloid beta peptide, universally known for its role in Alzheimer's disease. ConA aggregation has been monitored by static and dynamic light scattering, Thioflavin T emission and FTIR absorption and the morphology of different aggregates was verified by means Atomic Force Microscopy and Confocal Microscopy. Our results indicated that this protein undergoes aggregation through two distinct pathways modulated by pH and leading, respectively, to the formation of amyloids or amorphous aggregates. The relative weight of the two pathways is determined by pH: amorphous aggregates are formed preferentially at low pH values, close to the isoelectric point while fibrils formation is favoured at high pH values. In particular, we also showed that fibrillar structures are also formed close to physiological conditions. Con A fibril formation takes places from partially destabilized protein structures whose association involves β-aggregates structures, these intermediates interact to each other trough non-cooperative mechanism leading to the formation of macroscopic fibrillar aggregates.
Moreover we studied the effect of extracellular addition of Con A on human neuroblastoma LAN5 cells, the protein was added to the cell culture medium in its native state and in aggregated form and it was found to cause cells death via an apoptotic pathway. In particular, oligomeric aggregates were found to produce dose dependent highly toxic effects, whilst large fibrillar aggregates were found to be substantially harmless to the cells.

Poster No. II-25

EFFECTS OF HSP90 INHIBITORS ON TAU HYPERPHOSPHORYLATION IN NEURONS

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The molecular chaperones have been implicated in numerous neurodegenerative disorders in which the defining pathology is misfolded proteins and the accumulation of protein aggregates. In Alzheimer's disease (AD), the tau protein dissociates from microtubules and forms aggregates of hyperphosphorylated tau protein termed neurofibrillary tangles (NFT), which are neuropathological hallmarks of the disease. Misfolded proteins can undergo refolding and stabilization mediated by the 90 kDa heat shock proteins (HSP90). Inhibitors of HSP90 trigger HSP90 complexes inter alia with HSP70 and chaperone carboxyl terminus of Hsc70-interacting protein (CHIP) driving misfolded proteins such as phosphorylated tau species towards proteasome-mediated degradation.
We established neuronal cultures from wildtype mice and characterized different cell-permeable HSP90 inhibitors, which displayed a concentration-dependent upregulation of HSP70 and HSP40 in the 100-1000 nM range. The HSP90 inhibitors exhibited no cytotoxicity in primary neurons at the highest concentration (10 µM) tested. We observed significant reduction of tau phosphorylation at phospho-specific epitopes shown to be phosphorylated in AD brains without affecting the total tau protein expression in neurons. The HSP90 inhibitors were effective on HSP70 induction and reduction of phosphorylated tau in a similar concentration range. Additionally, we characterized HSP90 inhibitors in CHO cells overexpressing human mutant tau (TauP301L). Here, we observed a substantial decrease of total and phosphorylated mutant tau in a concentration dependent manner with the HSP90 inhibitors. Our results suggest that HSP90 inhibitors may indeed help neurons to clear misfolded proteins such as hyperphosphorylated tau that contribute to the pathogenesis of AD.

Poster No. II-26

CAN THE SAME PROTEIN BE AN AMYLOID PRECURSOR AND AN AMYLOID INHIBITOR? THE LESSON OF TRANSTHYRETIN (TTR).

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The offspring of crosses of APP23 mice, a robust model of human Aβ deposition, and mice highly over expressing wild type human TTR show less hippocampal and cortical $A\beta_{1-40}$ and $_{1-42}$ deposition and measures of spatial learning and memory that do not differ from control mice. When APP23 mice are bred to animals that have both endogenous *TTR* genes silenced cortical and hippocampal Aβ deposition appear much earlier than in controls. A small proportion of neurons in normal mice stained with an antibody to TTR but in APP23 animals many more neurons were stained as were the extracellular plaques. When the same antibody was used to stain brains from Alzheimer patients and non-demented age matched control individuals 70% of hippocampal neurons were positive in AD brains and only 10% in controls. Surface Plasmon resonance demonstrated that both human (previously shown by others) and murine TTR bound Aβ fibrils and monomers with the mouse protein having a higher

affinity for both. The data suggested that the in vivo beneficial activity was due to a direct interaction of TTR with some form of Aβ. In an effort to elucidate the mechanism and site of the interaction we have now shown that primary neurons from APP23 animals transcribe the TTR gene and synthesize the protein and that TTR inhibits Aβ- induced neuronal cytotoxicity. Hence in these animals TTR is a systemic amyloid precursor and a CNS amyloid inhibitor, a conundrum for those ascribing amyloidogenicity to only protein structure. It is also apparent that animals transgenic for the wild type forms of other amyloid precursors can suppress the Aβ phenotype in AD models raising additional questions regarding the specific structural properties of these proteins.

BENZON SYMPOSIUM No. 56

FUNCTIONAL AND PATHOGENIC PROTEIN AGGREGATION

AUGUST 24 – 27, 2009, COPENHAGEN, DENMARK

Organizing committee:

Daniel Otzen (Aarhus), Poul Henning Jensen (Aarhus), Sven Frøkjær (Copenhagen) & Niels Borregaard (Copenhagen)

Abstracts - WEDNESDAY, August 26, 2009

SYNAPSE PATHOLOGY AND THERAPEUTIC STRATEGIES IN SYNUCLEINOPATHIES

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Synaptic damage is an ealy event in the pathological process in synucleinopathies. Neurodegeneration in such conditions including combined forms of Alzheimer's and Parkinson's Disease might be related to the abnormal accumulation and interactions between Abeta and α–synuclein at the synapses. Alterations in the balance between factors promoting aggregation, clearance and synthesis of Abeta and α–synuclein might be centrally involved in the formation of oligomers and the pathogenesis of neurodegeneration. Studies in vitro and in APP and α–synuclein transgenic animal models have shown that Abeta and α–synuclein can interact leading to the formation of hybrid oligomeric complexes with a pore like structure. These channel like structures damage the neuronal membrane and might lead to abnormal ion flux. The mechanisms through which these oligomers lead to neurodegeneration include the formation of pores with abnormal calcium flux, mitochondrial dysfunction and alterations in the lysosomal dependent autophagy pathway. Accumulation of toxic oligomers occurs at early stages most likely in the synapse later progressing to the axon and neuronal cell body. Neuroprotective strategies directed at reducing the formation of synuclein oligomers and abnormal calcium fluxes might be critical in developing new treatments for DLB and PDD.

This work was done with NIH support (AG18440).

PATHWAYS IN ALPHA-SYNUCLEIN AGGREGATE TOXICITY

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Intracellular alphα-synuclein (AS) aggregates are a common finding in neurodegenerative disorders and a large body of evidence support that aggregated AS species significantly contributes to the degenerative process.

Pivotal questions concerns what forms of AS elicit the toxicity and how they harm the cell at the molecular level?

In a recently developed cell model we have shown been able to induce a slowly degenerative phenotype by the coexpression of AS and the stimulator of AS

aggregation p25α. The degeneration can be abrogated by several inhibitors of AS aggregation and also depend on the phosphorylation of Ser129. The model have allowed the further dissection of subcellular pathways with respect to cytosolic vs. nuclear localization and the analysis of early transcriptional responses to the development of intracellular AS aggregates.
These results will be discussed in relation to others models of AS toxicity.
References:
1. Kragh-CL et al., 2009 JBC 284;10211

MODIFYING ALPHA-SYNUCLEIN AGGREGATION IN LIVING CELLS

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A common pathological feature among neurodegenerative diseases is the excessive accumulation of aggregated proteins in the brain. In Parkinson's disease, the protein alpha-synuclein misfolds and is found in inclusions called Lewy bodies, the pathological hallmark of the disease. Misfolded proteins enable aberrant interactions with themselves and with other proteins, culminating with the disruption of the normal function of specific neuronal cells and, ultimately, their death. The formation of inclusions is preceded by the formation of smaller oligomeric species but, at the present moment, it is still unclear which of those species is most toxic.
Thus, a complete picture of alpha-synuclein misfolding, aggregation, and clearance will be crucial for the understanding and treatment of synucleinopathies.
In this study we seeked to identify genetic modifiers of alpha-synuclein oligomerization using a genome-wide knock down screen using the novel bimolecular fluorescence complementation (BiFC) assay as a readout of alpha-synuclein dimerization/oligomerization in living cells. The identified genes will be tested in animal models of Parkinson's disease and, ultimately, enable the development of novel therapeutic strategies, relevant not only for familial but also for sporadic cases of synucleinopathies.

MISFOLDING OF PROTEINS WITH A POLYGLUTAMINE EXPANSION IS FACILITATED BY PROTEASOMAL UNFOLDASES

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Deposition of misfolded proteins with a polyglutamine expansion is a hallmark of Huntington disease and other neurodegenerative disorders. Impairment of the proteolytic function of the proteasome has been reported to be both a cause and a consequence of polyglutamine accumulation. We found that the proteasomal chaperones that unfold proteins to be degraded by the proteasome but also have non-proteolytic functions co-localized with huntingtin inclusions both in primary neurons and in Huntington disease patients and formed a complex independently of the proteolytic particle. Overexpression of Rpt4 or Rpt6 facilitated aggregation of mutant huntingtin and ataxin-3 without affecting proteasomal degradation. Conversely, reducing Rpt6 or Rpt4 levels decreased the number of inclusions in primary neurons, indicating that endogenous Rpt4 and Rpt6 facilitate inclusion formation. In vitro reconstitution experiments revealed that purified 19S particles promote mutant huntingtin aggregation. When fused to the ornithine decarboxylase destabilizing sequence, proteins with expanded polyglutamine were efficiently

degraded and did not aggregate. We propose that aggregation of proteins with expanded polyglutamine is not a consequence of a proteolytic failure of the 20S proteasome. Rather, aggregation is elicited by chaperone subunits of the 19S particle independently of proteolysis.

REDIRECTING AMYLOID FORMATION PATHWAYS WITH SMALL MOLECULES

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Amyloid diseases are a large group of protein misfolding disorders, which include Alzheimer's, Parkinson's, Huntington's disease and forms of ataxia. They are characterized by the accumulation of protein aggregates in tissues or the extracellular matrix, which has detrimental consequences for the cellular system. An increasing body of evidence indicates that soluble amyloid oligomers and ß-sheet-rich protofibrils, rather than mature fibrils or amyloid plaques, are the major toxic species that cause dysfunction and neurodegeneration. An exact characterization of the pathogenic molecules and the cellular processes they are involved in, however, is still missing. We have been using cell-free and cell-based model systems to investigate the effects of small molecules and proteins on the different species in the amyloid formation cascades of huntingtin, α–synuclein or amyloid-ß. Both accelerators and inhibitors of amyloidogenesis were identified. Moreover, small molecules were discovered that directly bind to natively unfolded proteins and efficiently redirect amyloid assembly pathways. Chemical compounds and proteins can interfere with different steps in the amyloid formation cascade and are useful tools for protein misfolding research.

THERAPEUTIC STRATEGIES FOR INHIBITING THE GENERATION OR ENHANCING THE CLEARANCE OF PROTEIN AGGREGATES FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

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There is a growing body of evidence that an increased propensity for proteins to misfold can give rise to neuronal dysfunction and neurodegeneration by generating oligomeric, fibrillar, and higher-order aggregates such as amyloid plaques, neurofibrillary tangles, Lewy bodies, and huntingtin inclusions. Hence protein misfolding and aggregation appears to be a common theme in the pathogenesis of neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and Huntington's disease. Some of the earliest steps in the pathology of these diseases appears to involve the generation of soluble oligomers of proteins and peptides such as the APP cleavage product Aβ, prior to the generation of amyloid plaques, the pathological aggregation of phosphorylated Tau protein believed to eventually lead to neurofibrillary tangles, and the generation of soluble synuclein oligomers eventually leading to Lewy bodies in the progression of Parkinson's disease. Reducing or eliminating early steps during such protein aggregation may represent an attractive therapeutic target for neurodegenerative disorders. This presentation will provide an overview of some of the approaches being used in preclinical and clinical studies seeking to lower brain and CSF levels of Aβ oligomers. Such approaches include the prevention of oligomer generation by inhibition of BACE and gamma secretase, the two enzymes generating Aβ, passive immunotherapy approaches to lower a range of

Aβ species, and approaches aimed at enhancing the proteolysis of aggregated Ab, such as inhibition of plasminogen activator inhibitor-1. The clinical validation of such approaches would represent a major milestone in the development of disease modifying treatments for neurodegenerative disorders.

Poster No. III-1

IS PROTEIN MISFOLDING PAYHOLOGY PROPAGATED FROM ONE NEURON TO ANOTHER IN THE PARKINSON'S DISEASE BRAIN?

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In Parkinson's disease (PD), aggregates of alphα-synuclein in the cytoplasm and neurites are classical neuropathological findings. These Lewy neurites and Lewy bodies are prominent in the substantia nigra, but also found in widespread areas throughout the nervous system. During the past year, a series of papers have shown that classical neuropathological signs of PD appear in grafted embryonic neurons (1, 2, 3). A subset of grafted cells display several of the classical features of Lewy bodies, including Thioflavin S staining and immunoreactivity for alphα-synuclein which is phosphorylated at serine residue 129. From 2 to 5% of the grafted dopaminergic neurons display Lewy bodies (apparently the frequency increases with time), and they first appear around one decade after surgery. Other cells in the grafts exhibit signs associated with aging (e.g. increased cytoplasmic staining for alphα-synuclein) and partial functional impairment (e.g. reduced levels of VMAT2). Despite these changes in the grafts, some PD patients with transplants still exhibit signs of functional recovery even beyond a decade after grafting. In my presentation, I will discuss possible mechanisms underlying the transfer of pathology to the young grafted neurons and whether they are relevant to our understanding of how neuropathology normally spreads in the PD brain (4).

References:

1. Li et al. (2008) Lewy bodies in grafted neurons in subjects with Parkinson's disease suggest host-to-graft disease propagation. Nat Med 14: 501-3.
2. Kordower et al (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson's disease. Nat Med 14: 504-6.
3. Kordower et al (2008) Transplanted dopaminergic neurons develop PD pathologic changes: a second case report Mov Disord 23: 2303-6
4. Brundin et al (2008) Research in motion: the enigma of Parkinson's disease pathology spread. Nat Rev Neurosci 9: 741-5.

Poster No. III-2

C-TRUNCATED ALPHA-SYNUCLEIN TRIGGERS TOXICITY OF FULL LENGTH ALPHA-SYNUCLEIN *IN VIVO*

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Lewy bodies, pathological hallmark of Parkinson's disease, are form by insoluble polymers of alphα-synuclein (α–syn). The fibril formation is preceded by oligomeric forms of the protein that have been suggested to be the toxic species in the disease. Among the different modification that can promote the formations of oligomeric α–syn, C-terminal truncations are one of the most abundantly found in PD patients. In

vitro, C- terminal truncated α–syn aggregates faster and sub-stoichiometric amounts of C-terminal-truncated α–syn promoted aggregation of the full length α–syn (α–synFL) and induced neuronal toxicity. To address *in vivo* the putative enhancement of α–syn toxicity in the presence of low amount of truncated α–syn, we use recombinant viral vectors to achieve sub-threshold expression levels of either α–synFL or a C-terminal truncated α–syn (1-110) (α–synΔC) alone or in combination in adult rat midbrain. Sub-threshold levels of α–synFL induced no cell death and little pathological accumulation of α–syn in dopaminergic cells of the striato nigral pathway. Neither α–synΔC at low levels was able of inducing dopaminergic cell death although numerous pathological accumulations of α–syn were observed in striatal dopaminergic fibers. However, when α–synΔC and α–synFL were co-express at those sub-threshold levels animals showed progressive accumulation of α–syn, loss of dopaminergic fiber density at striatal level, neuronal cell loss in SN and motor abnormalities consistent with dopaminergic dysfunction. Therefore we show that *in vivo* C-terminal truncated enhances α–syn aggregation and neurotoxicity on dopaminergic cells.

Poster No. III-3

INTRACEREBRAL INOCULATION OF PRION-INFECTED BRAIN HOMOGENATE WITH A BETA-SHEET PEPTIDE (RADA16) DISRUPTS PRION ACCUMULATION AND EXTENDS HAMSTER SURVIVAL

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Transmissible spongiform encephalopathies are incurable, fatal neurodegenerative diseases characterized by the accumulation of abnormal prion protein (PrPsc), neuronal cell death and vacuolation of brain tissue. The PrPsc protein is extractable from diseased tissue and is distinguished from endogenous PrPc by partial protease resistance and detergent insolubility. The transmissible agent is the PrPsc protein that serves as a template for the molecular conversion of endogenous host PrPc into the abnormal PrPsc structural isoform. Host expression of PrPc is necessary for disease transmission, as ablation of the PrPc gene prevents disease. The molecular events that mediate neuronal PrPc to PrPsc conversion, not simply accumulated PrPsc, appears to be the initiating factor mitigating the neurodegenerative disease process. A multitude of synthetic peptides have been designed to exploit defined protein domains and shown to facilitate a variety of cellular processes. The RADA-16 peptide repeat (RADA) has been shown to self-assemble into a nanofiber hydrogel composed of anti-parallel beta-sheets. A hallmark of aggregate amyloid proteins appears to be the predominance of the anti-parallel beta-sheet protein structure. Indeed, prion infection promotes the conversion of the alpha-helical PrPc into an abnormal PrPsc rich anti-parallel beta-sheet structure. Accumulation and aggregation of prion amyloid is likely mediated by repeated stacking of prion anti-parallel beta-sheets. We hypothesize that the anti-parallel beta-sheet structure of RADA can interact with those of PrPsc and disrupt prion accumulation. We show that intracerebral inoculation of PrPsc with RADA significantly delays disease onset and increases hamster survival. Time of survival was dependent on the dose of RADA and pre-incubation with PrPsc prior to inoculation. RADA treatment results in the absence of detectable PrPsc at 40 d as compared to control and this was followed by an increased rate of PrPsc accumulation until the time of sacrifice. In all PrPsc-inoculated animals, clinical symptoms were observed ~10 d prior to sacrifice and brains showed

spongiform degeneration with Congo red positive plaques. A time-dependent increase in reactive gliosis was observed in both groups with more GFAP detected in RADA-treated animals at all time points. The PrP protein showed dose-dependent binding to RADA and this binding was competitively inhibited by Congo Red. We conclude that RADA disrupts the efficacy of prion transmission by altering the rate of PrPsc accumulation. This is the first demonstration that a self-assembling biomolecular peptide can interact with PrPsc, disrupt the course of Scrapie disease process, and extend survival.

Poster No. III-4

DIRECT EGCG-INDUCED REMODELING OF PREFORMED BETA-SHEET RICH AMYLOID FIBRILS REDUCES TOXICITY

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Recently, we demonstrated that the polyphenol (-)-epigallocatechingallate (EGCG) inhibits α–synuclein (aS) and amyloid-β (Aβ) fibrillogenesis by associating with natively unfolded polypeptides and promoting the self-assembly of unstructured oligomers of a new type (Ehrnhoefer & Bieschke et al. Nature Struct. Mol. Biol. 2008), which may open a new route to the prevention and therapy to Alzheimer's disease, Parkinson's disease, and other amyloidoses. However, whether EGCG also disassembles mature amyloid fibrils remains unclear.

Here, we show that EGCG directly binds both to mature αS and Aβ fibrils and converts them into amorphous protein aggregates that are non-toxic for mammalian cells. The conversion of amyloid fibrils was followed in vitro by electron microscopy and atomic force microscopy, loss of secondary structure was monitored by circular dichroism, loss of the competence to propagate amyloid formation, i.e. 'molecular infectivity', was demonstrated in seeding assays. Remodeling of αS and Aβ fibrils removed their toxicity to neuronal model cells (rat pheochromocytoma, PC12).

We determined the molecular mechanism of the remodeling process by fluorescence microscopy experiment in which amyloid fibrils were mixed, which had been uniformly labeled by incorporation protein monomers attached to a red or a green fluorophore.

Contrary to expectation and contrary to what has been observed previously for anti-amyloid drugs, our results demonstrate that the fibrils are not disassembled into small oligomers or monomers in the conversion process, but rather that the remodeling process occurs directly on the amyloid fiber. Direct remodeling may thus prove an unexplored drug strategy to detoxify amyloid deposits at a late disease stage, without trying to reverse the aggregation process.

Poster No. III-5

DISSECTING THE MECHANISMS OF NON-SPECIFIC INHIBITORS OF PROTEIN AGGREGATION

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Increasing evidence indicates that the misfolding and aberrant self-assembly of proteins is often associated with the molecular events leading to neuronal death in a range of neurodegenerative diseases. The limited understanding of the amyloid aggregation phenomenon, together with the intrinsically disordered nature of many of these proteins, make it particularly challenging to develop effective strategies for discovering small molecules inhibitors of their aggregation. Interestingly, some small molecules have been reported to interfere with the misfolding of several amyloidogenic proteins, thus representing potential general anti-amyloid agents. We present here a multidisciplinary biophysical approach to dissect the interaction mechanisms of two such compounds with the protein α–synuclein, whose amyloid-like aggregation is closely connected with Parkinson's disease. By employing low and high resolution spectroscopic techniques, together with calorimetry, dynamic light scattering, fluorescence and computer-aided docking we are capable of providing a detailed understanding of the anti-amyloid activities of the compounds. We show how these small molecules interact with certain regions of the protein with different extent and affinities and the consequent inhibitory effects on the aggregation of the protein. The mechanistical differences observed in binding and inhibition of amyloid fibril formation may be attributable not only to the chemical nature of the small molecules but also to their abilities for self-assembly. These findings provide potential explanations for the non-specific anti-amyloid effect obeserved for these compounds as well as important mechanistical information for future drug discovery efforts targeting the misfolding and aggregation of intrinsically unstructured proteins.

Poster No. III-6

THE EFFECT OF THE LIPID PEROXIDATION METABOLITE 4-OXO-2-NONENAL ON THE STRUCTURE AND FUNCTION OF ALPHA-SYNUCLEIN

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The aldehyde 4-oxo-2-nonenal (ONE) was recently identified as a product of lipid peroxidation and found to modify proteins effectively. Herein, we investigated structural implications of the interaction between ONE and α–synuclein, a protein which forms intraneuronal aggregates in neurodegenerative disorders such as dementia with Lewy bodies and Parkinson's disease. Our results show that ONE induced an almost complete conversion of monomeric α–synuclein into 40–80 nm wide and 6–8 nm high soluble β-sheet-rich oligomers with a molecular weight of about 2000 kDa. Moreover, the oligomers were very stable and not sensitive to treatment with sodium dodecyl sulphate, indicating that ONE cross-links individual α–synuclein molecules. Finally, the ONE-induced oligomers caused increased toxicity in a HEK-293 cell line compared to control treated cells.

Poster No. III-7

ALPHA-SYNUCLEIN OLIGOMER BINDING PROTEINS

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The natively unfolded protein α–synuclein is the major protein in proteinous inclusions, called Lewy bodies, which are found in patients diagnosed with Parkinson's disease [1]. α–Synuclein has the propensity to aggregate and on the

pathway to form mature fibrils, several intermediate structures exist [2]. A currently accepted hypothesis is that the cytotoxic form is the oligomeric forms of α–synuclein. Ligands binding to α–synuclein oligomers, and not or in less degree to the monomeric or fibrillary structure, may be responsible for this cytotoxicity. The oligomer binding ligands are isolated by immunoprecipitation and identified by gel electrophoresis followed by mass spectrophotometric analysis. Several ligands are identified including Parkinson's disease related proteins such as p25α. The on-going experiments are supported by the Michael J. Fox Foundation - RRIA.

References:
1. Volles MJ & Lansbury PT. Jr., Zeroing in on the Pathogenic Form of α-synuclein and its Mechanism of Neurotoxicity in Parkinson's Disease. Biochemistry, 2003.42(26): p.7871-7878
2. Fink AL., The aggregation and fibrillation of α-synuclein. Acc Chem Res, 2006. 39(9): p. 628-634

Poster No. III-8

9,10-ANTHRAQUINONE INTERFERES WITH Aß-PEPTIDE AGGREGATION: MECHANISM OF ACTION

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Amyloid aggregation is implicated in various neurodegenerative syndromes including Alzheimer's disease (AD). In this pathology, the β-amyloid peptides (Aβ) assembly into oligomeric aggregates, protofibrils, fibrils and, ultimately, into amyloid plaques, which represent the characteristic hallmark of the AD. Despite several low molecular weight compounds impairing Aβ aggregation are currently known, a detailed description of their interactions with Aβ oligomers and fibrils is hitherto missing. In the present work, the effect of two relatively similar compounds (i.e., 9,10-anthraquinone (AQ) and anthracene (AC)) on the early aggregation of the peptide stretch, promoting the Aβ self-assembly (i.e., $H_{14}QKLVFF_{20}$), is investigated through molecular dynamics simulations. Our results suggest a mechanism of activity for AQ, compared to AC, that consists in the strong binding interaction with $Aβ_{14-20}$ leading to the disruption of inter-strand hydrogen bonds and, thus, in destabilizing the peptide backbone organization. These findings are further experimentally confirmed by thioflavin T binding assay indicating the primary role of AQ in reducing the amount of aggregated $Aβ_{1-40}$ peptide. In this scenario, the atomic level description of the interactions between AQ and Aβ oligomers can represent a solid preliminary basis for the design of small-molecule inhibitors of aggregation having therapeutic potential in Alzheimer's disease.

References:
1. M. Convertino, R. Pellarin, M. Catto, A. Carotti and A. Caflisch; *9,10-Anthraquinone hinders β-aggregation: How does a small molecule interfere with Aβ-peptide amyloid fibrillation?* Protein Sci*, in press*

Poster No. III-9

ROLE OF MOLECULAR CHAPERONES IN SUPPRESSING THE AGGREGATION OF ALPHA -SYNUCLEIN

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Heat shock proteins have been shown to be protective in a number of neurodegenerative diseases (Auluck *et al.*, 2002). Considerable work has been done

on Hsp70 in this regard: Klucken *et al.,* 2004 demonstrated that Hsp70 can reduce the amount of misfolded aggregated alphα-synuclein species *in vivo* and *in vitro* and that is also protects cells from alphα-synuclein dependent toxicity. Molecular chaperones by their very nature are expected to target exposed hydrophobic surfaces such as those present in alphα-synuclein (Muchowski *et al.,* 2000). In contrast to Hsp70, however, very little is known about the action of other molecular chaperones on protein aggregation, particularly Hsp90 and its co-chaperones, despite the fact that Hsp90 is known to co localise in Lewy Bodies associated with alphα-synuclein aggregation (Uryu K., Am J Path, 2006 March).
Hsp90 is known to work in conjunction with various co-factors, co-chaperones and other molecular chaperones in the form of a cellular assembly machine. This large chaperone complex is essential for the correct maturation and activation of key client proteins. We are investigating the effects of chaperones Hsp90, Hsp70 and Hop alone and in combination on alphα-synuclein aggregation. Methods such as ThioflavinT fluorescence binding assays and a quartz crystal microbalance (QCM) are being used to study the aggregation process. Preliminary results are promising and establish that both Hsp90 and Hop, both have strong inhibitory activities. Further work is currently underway aimed at elucidating the mechanism by which these molecular chaperones prevent the aggregation of alphα-synuclein.

References:
1. Pavan K Auluck,H.Y Edwin Chan, John Q Trojanowski, Science, Feb 2002,295, 865-868
2. Jochen Klucken, Youngah Shin, Eliezer Masliah, Bradley T Hyman,Pamela J.Maclean, J.Biol.Chem,Vol 279,No 24,25497-25502
3. Clark J.I, Muchowski P.J, Curr.Opin. struct. Biol.:10,52-59
4. Uryu K, Richter-Landsberg C, Welch W, Sun E, Goldbaum O, Norris EH, Pham CT, Yazawa I, Am J Path,2006 March;168(3)947-61

Poster No. III-10

STUDY OF THE ROLE OF ALPHA-SYNUCLEIN PHOSPHORYLATION AT SER 129 IN DOPAMINERGIC NEURODEGENERATION IN PARKINSON'S DISEASE

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Studies during the last decade have established a central role in Parkinson's disease (PD) neurodegeneration for the protein alphα-synuclein (α–syn). Some of the factors that could affect α–syn toxicity in PD are post-translational modifications that may lead to changes in function or interaction with other proteins. Among the different post-translational modifications that human α–syn can undergo, oxidation, truncation and phosphorylation have been related to the disease.
α–syn in Lewy bodies has been found to be phosphorylated at Ser 129 and this phosphorylation seems to be related to the disease progress in PD, since S129P-a-syn is found in low levels in healthy humans and it is related to increased inclusion formation. In order to elucidate the role of phosphorylation of α–syn at Ser 129 in the disease process we used a rat PD model based on the local injection of recombinant adeno-associated viral vectors (rAAV). We created mutations in α–syn at Ser129, replacing the serine either to alanine (S129A) to block phosphorylation or to aspartate (S129D) to mimic phosphorylation. We overexpressed wt, S129A and S129D α–syn in rat midbrain by using rAAV and thereafter have compared the effect of the overexpression on dopaminergic neurons at different time points (at 4-8-15 weeks) post-injection. Our results show that pseudophosphorylated α–syn S129D induced faster motor deficit compared to S129A. However both S129D and S129A α–

syn induced decrease of dopaminergic cells in substantia nigra and pathological accumulations of α–syn in the striatal dopaminergic fibers. On our hands, the phosphorylation of a-syn at Ser129 seems to have a role in the progression of the disease but is not necessary for pathology, motor deficits or cell death to occur. Therefore our results differ from those obtained previously in a PD drosophila model where avoidance of phosphorylation at Ser129 was sufficient to prevent cell death of dopaminergic neurons.

Poster No. III-11

α–SYNUCLEIN INTERACTION WITH DETERGENTS

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Parkinson's Disease (PD) is a common neurodegenerative disorder of the brain associated with death of dopaminergic neurons. Lewy Bodies (LBs) are observed in the region of dopaminergic neurons and the main constituent of LBs is the 14.4 kDa natively unfolded protein alpha synuclein (αSN) and its aggregation has been linked tightly to PD.
In this study we focus on the aggregation of αSN in the presence of sodium dodecyl sulphate (SDS), a well-known lipid mimicking detergent. We use Far-UV Circular Dichroism (Far-UV CD) for detection of conformational transitions induced by SDS, isothermal calorimetry (ITC) for binding curves of SDS to αSN, Thioflavin T binding for following aggregation of αSN at different SDS concentrations, as well as electron microscopy (EM) for visualizing the aggregates.
The data we present shows a multistate nature of αSN folding/aggregation, where αSN will fold upon SDS addition from random coil to α–helix and the transition is dependent upon micelle formation of SDS. We assign an aggregation optimum for SDS: αSN and demonstrate that the aggregation propensity induced by SDS is highly dependent upon the micelle formation.

Poster No. III-12

IRON-MEDIATED TOXICITY IN DOPAMINERGIC, NEURONAL CELL CULTURES: IMPLICATIONS FOR THE PATHOGENESIS OF PARKINSON'S DISEASE

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Both α–synuclein and increased levels of iron are probable parameters involved in Parkinson's disease (PD) pathology in the affected substantia nigra pars compacta (SNpc). Iron participates in Fenton chemistry causing the formation of reactive oxygen species (ROS) that may cause oxidative stress, eventually resulting in oxidative damage to proteins. This study investigated the effects of two iron-compounds on ROS formation in cultured dopaminergic neurons expressing wild-type, A53T or A30P α–synuclein. Ferric ammonium citrate (FAC) proved a relevant Fe-compound in experimental modeling of oxidative stress as determined by free radical formation. Increasing concentrations of FAC also lead to oxidative damage as indicated by formation of malondialdehyde (MDA) in homogenates of lysed cells. The FAC exposure also leads to formation of thioflavin-containing aggregates; these

aggregates predominantly occurred in cells with A53T and A30P mutations of α–synuclein. Another iron compound, $FeCl_2$, commonly used for causing oxidative damage due to cellular iron-accumulation, hence modeling the condition of nigral pathology in PD, exerted much larger scale levels of oxidative stress and damage than that of FAC, and was much more prone to cause formation of thioflavin-containing aggregates, even in wild-type dopaminergic neurons. However, $FeCl_2$ also precipitated on the surface of the dopaminergic neurons. Hence, the higher toxicity of $FeCl_2$ was probably not only due to exaggerated formation of reactive oxygen species because of cytosolic accumulation, but probably also due to changes of the cell membrane integrity as a resultant of iron-hydroxide formation via direct interaction of extracellular $FeCl_2$ with lipids of the cell membrane. These observations emphasizes the importance of iron and oxidative stress in PD pathology, as low molecular weight iron compounds are taken up by dopaminergic neurons and cause free-radical formation in particular in A53T and A30P mutant neurons.

Poster No. III-13

STRUCTURE AND STABILITY OF WT PARKIN AND ITS VARIANTS R275W AND T240R: NEW INSIGHTS INTO PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disorder and remains incurable. Considerable progress has been made in understanding the molecular mechanisms underlying this pathology, in particular with the identification of several genes linked to familial forms of PD. (1). Among them, *PARK2* encodes for parkin, an E3 ubiquitin ligase which might act as a multipurpose neuroprotective agent (2). Single heterozygous polymorphisms in the parkin gene are of particular interest due to the largely unknown frequency of these substitutions in healthy individuals that may contribute to early onset parkinsionism (3). The absence of parkin activity, induced by loss-of-function point mutations, is expected to cause neurodegeneration due to the accumulation of one or several of its substrates in dopaminergic neurons, especially in the substantia nigra. In the present study, we investigated whether WT parkin and the variants R275W and T240R lead to the accumulation of misfolded proteins as a consequence of a severe structural modification. A detailed comparison of the conformational properties of the WT and mutant proteins, combining biophysical and biochemical methodologies, was undertaken. The present work contributes to a better understanding of how point mutations affect parkin structure and stability providing new insights into the molecular basis of PD.

References:

1. Thomas, B., and Beal, M. F. (2007) Hum Mol Genet 16 Spec No. 2, R183-194
2. Feany, M. B., and Pallanck, L. J. (2003) Neuron 38, 13-16
3. Dawson, T. M. (2006) J Neural Transm Suppl, 209-213

Poster No. III-14

EFFICIENT REDUCTION OF ABETA LOAD IN TRANSGENE APP MICE WITH Aβ42 SPECIFIC VACCINE

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Alzheimers Disease (AD) is characterised by the accumulation of neurotoxic forms of aggregated (plaques) and oligomer forms of Abeta peptides in the brain of AD patients. Previous vaccine trials used the non-modified target protein, Aβ42, as immunogen to prevent and reduce the Abeta load in AD patients. Although one trial showed evidence of plaque clearance, indicative of a therapeutic effect on the neuropathology, the trial was terminated when 6% of the patients were diagnosed with meningo-encephalitis. The cause of the meningo-encephalitis remains unknown but subsequent analyses have been put forward: 1) Activation of Aβ42 specific T-cells that migrates to the brain , 2) strong adjuvant initiating a Th1 response.
Here we show that presence of strong T cell helper epitopes fused with Aβ42 suppress the T cell response to the weaker self-epitopes present in the Aβ42, presumably by immunodominance. The immunogen comprising Aβ42 and helper T cell epitopes induce higher Aβ42 specific antibody titers in comparison to unmodified Aβ42 and is well tolerated in mice, Guinea pigs and monkeys. The AD animal model Tg2576, which is transgenic for APP, showed clear reduction of plaque formation and plasma Aβ42 load when treated with this immunogen. We conclude that immunodominant peptides associated with Aβ42 inhibit activation of Aβ42 specific T cells and yet induce an effective and specific antibody response which reduces the Aβ42 load.

Poster No. III-15

ROLE OF MICROGLIA IN PARKINSON'S DISEASE: ALPHA-SYNUCLEIN AS INITIATOR OF ACTIVATION

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Post-mortem brain analysis from Parkinson's disease patients strongly support microglia activation and the adaptative immune response as factors contributing to disease progression. Different evidences in-vitro and in-vivo suggest that alpha-synuclein may trigger such responses. To investigate this we used a recombinant viral vector to express human alpha-synuclein in rat midbrain at levels that none or significant cell death in the SN was induced. Microglia activation was assessed by stereological quantification of the number and morphology of Mac1+ cells, as well as CD68 and MCH-II immunoreactivity. Stereological analysis of Mac1+ microglia, indicate that in the presence of alpha-synuclein induced cell death, there is a robust increase in numbers at 8 weeks that is not observed in the alpha-synuclein expressing animals where cell death was absent. Furthermore, in the occurrence of cell death, there was a significant increase and long-lasting elevated CD68+ expression. Contrastingly, in the absence of cell death, a significant robust early and persistent up-regulation of MHC II was observed. T-lymphocite infiltration, as judge by the presence of CD3+ cells, was observed in animals expressing alpha-synuclein independently of the presence of cell death. Our results suggest that the microglial

response to human alphα-synuclein differs in the presence or absence of cell death, but it is not restricted to only one of the conditions, indicating that microglia activation can be initiated by early events related to alphα-synuclein over-expression different than cell death.

Poster No. III-16

CHEMICALLY MODIFIED APTAMERS APPLIED TO TARGET THE FORMATION OF ALPHA-SYNUCLEIN FIBRILS

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Neurodegenerative diseases are characterized by the accumulation of insoluble protein aggregates, dominated by a single protein specific for the disease in question. In the case of Parkinson's disease, this is the 140-residue α–synuclein that forms fibrils with indefinite length and a width of a few nm, a process that also can occur with isolated protein in vitro. The fibrils are the end-result of a long and complex process, which at the early stages involves the formation of ring-shaped oligomers. The development of Parkinson's disease in a patient correlates with the formation of α-synuclein aggregates, so called Lewy Bodies, within brain dopaminergic neurons [1]. Current drugs only provide symptomatic relief for a limited period and new strategies are urgently needed. A key observation is the known ability of α–synuclein oligomers to cause small-molecule leakage of synthetic vesicles leading to cell death in vitro. This suggests that the oli! gomer is the toxic species and makes this an obvious drug target.

Systematic evolution of ligands by exponential enrichment (SELEX) is a relatively new approach for generating potential therapeutic and diagnostic agents [2]. The technique combines the ability of RNA or DNA oligonucleotides to fold into a variety of three-dimensional structures depending on their nucleic acid sequence, with the possibility of selecting from very large pools of random sequences ($\sim 10^{15}$). Aptamers are remarkable in terms of affinity and specificity. To acquire in vivo applicable RNA aptamers, selections can be performed with f.ex. fluoromodifications at the 2´ position of pyrimidines improving their stability in biological fluids significantly (JK-P6). Such RNA aptamers are good starting points for generating agents for diagnostic and/or therapeutic applications. Aptamers have shown low to no toxicity in vivo and unlike antibodies, no immunoreactivity. Additionally, the small size of aptamers (10-15 kDa) compared to antibodies (150 kDa), together with their polyanionic nature, results in good tissue penetration and rapid blood clearance.

Therefore applying aptamers techonology to purified α-synuclein oligomers as targets should increase the chances of selecting an inhibitor of fibrilation. In another approach, monomeric α–synuclein under conditions where oligomers and fibrils are readily formed is also interesting target. By isolating aptamers bound specifically to monomeric α–synuclein not entering fibrils we will be able to specifically select early-stage inhibitors of aggregation.

References:

1. Lundvig D, Lindersson E, Jensen PH. Pathogenic effects of α–synuclein aggregation. Brain Res Mol Brain Res. 2005. 134(1): 3-17.
2. Que-Gewirth NS, Sullenger BA. Gene therapy progress and prospects: RNA aptamers. Gene Ther. 2007. 14(4): 283-91.

Poster No. III-17

CONFORMATIONAL STRUCTURE OF PRION PROTEIN AGGREGATES AS STUDIED BY HYDROGEN/DEUTERIUM EXCHANGE AND MASS SPECTROMETRY

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Transmissible spongiform encephalopathies are a group of fatal neurodegenerative disorders including 'mad cow' disease in cattle and Creutzfeldt-Jakob disease in humans. These diseases are believed to be caused by prions, a class of unique, protein-only infectious agents resulting from conformational conversion of normal, cellular form of prion protein (PrP^{C}) to amyloid-like, misfolded aggregate (PrP^{Sc}). While PrP^{C} has been well characterized by NMR spectroscopy, one of the most difficult challenges in the field is the determination of the structure of the infectious PrP^{Sc} conformer. Recent data indicates that PrP^{Sc}-like aggregates can be generated *in vitro* from the recombinant prion protein (rPrP) by 'protein misfolding cyclic amplification' (PMCA) reaction, where conformational conversion of rPrP is templated by brain-derived PrP^{Sc}. Here we have characterized conformational structures of these PMCA-generated aggregates (PrP^{PMCA}) of the recombinant Syrian hamster PrP and spontaneously formed amyloid fibrils (PrP^{SP}) using hydrogen/deuterium exchange coupled with mass spectrometry. Our data indicates that PrP^{PMCA} aggregates are heterogeneous and structurally different from PrP^{SP}. The main population of molecules is characterized by systematically H-bonded β-sheet core starting at residue ~145 and extending to the C-terminus. The other populations display shorter β-cores, with N-termini between residues 145 and 181. Furthermore, a fraction of molecules shows significant protection against hydrogen exchange within the region 117-133. Importantly, there is no systematic hydrogen bonding within the N-terminal part encompassing residues ~90-116, indicating the absence of any stable β-sheet structure within this region.

Poster No. III-18

BIOMARKER DISCOVERY THROUGH SECRETOME ANALYSIS OF PARKINSON'S DISEASE MODEL CELL LINES

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Parkinson's disease (PD), a chronic degenerative brain disease, is characterized by a loss of dopiminergic neurons in the substantia nigra area of the brain and the abnormal presence of ubiquitinated cytoplasmic inclusions called Lewy bodies in affected cells. Parkinson's disease can be diagnosed precisely by post-mortem analysis of the affected areas of the brain whereas early clinical diagnosis of PD may be based on the patient's medical history and neurological examinations before a symptom calming treatment can be implemented. Thus, the interest in finding early biomarkers for this neurodegenerative disease before clinical symptoms arise is great.

Cerebrospinal fluid (CSF) is the most relevant biological fluid for biomarker study because CSF has direct contact with the extra cellular space in the brain. The CSF Proteome is, however, extremely complex and patient samples are often available only in small volume whereby direct analysis by specific PD biomarkers is a major analytical challenge. Alternatively, Conditioned media obtained from appropriate PD

model cell lines enable identification of potential biomarkers for PD by investigation of expression profiles of secreted proteins and composition of micro vesicles.
We present here the methodology for purification and identification of secreted proteins and micro vesicle-associated proteins from PD cell lines that change the expression level as a consequence of overexpression of specific proteins (e.g. α–synuclein, Parkin or P25α) through a quantitative mass spectrometry based approach.

Poster No. III-19

MAPPING PROTEIN INTERACTION NETWORKS FOR NEURODEGENERATIVE DISEASES USING A MICROARRAY-BASED YEAST-TWO-HYBRID APPROACH

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A major research interest in our group is the systematic exploration of protein-protein interactions (PPIs) that are relevant for neurodegenerative diseases. Previously, our group has established a high-throughput yeast two-hybrid (Y2H) screening procedure using an array-mating technology. Here, we present a novel Y2H strategy that uses pooling and selective growth combined with readouts from DNA microarrays (Affymetrix platform). This method constitutes an alternative to the classical pair-wise Y2H interaction mating. In the typical experimental setup, a complete set of prey strains containing about 14,000 cloned human open reading frames is pooled, and aliquots are mated with individual bait strains. Mated yeast populations are then grown under selective conditions for Y2H interactions. After selection, cDNAs encoding prey proteins that interact with the baits are overrepresented in the populations and can be identified by hybridization to DNA microarrays. Hence, the novel microarray-based screening scheme allows a massive parallel processing of Y2H experiments and, importantly, the rapid elimination of false positive interactors in the screens. Selection of interactors in low volumes of liquid culture allows direct testing of drugs that target relevant PPIs, which is another aim of the project. Validation of the newly identified interactors is performed with automated luminescence-based mammalian interactome mapping (LUMIER) and by functional *in vivo* and *in vitro* assays. For the future, we expect that this novel approach will considerably accelerate the mapping of the entire human protein interactome.

Poster No. III-20

PREVENTION OF AMYLOID FIBRIL FORMATION BY A SMALL HEAT SHOCK PROTEIN

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Amyloid fibril formation is a potential risk factor for various pharmaceutically relevant peptides and proteins, such as glucagon, calcitonin, GLP-1 and -2, and insulin. Fibril formation can result in increased cost-of-goods as well as cause unwanted side-effects, such as immune responses.
In this project we investigated the ability of a small heat shock protein, α-crystallin, in preventing fibril formation of bovine insulin at physiological pH (pH 7.4). Even at insulin:α-crystallin molar ratios of 50:1 a significant reduction in fibrillation kinetics

was observed, while at 10:1 molar ratios the kinetics were slowed down by a factor 20. In contrast, the commonly used protein stabiliser human serum albumin (HSA) yielded only a modest reduction (factor 2) in fibrillation kinetics. α-Crystallin was also superior to mini-α-crystallin peptides in its prevention of fibril formation. In contrast to the fibrillation data, further biophysical studies showed that α-crystallin physically destabilizes the insulin hexamer, promoting dissociation to the fibrillation-prone monomeric species.
Taken together, these results indicate that alpha-crystallin prevents insulin fibrillation by binding to an on-pathway intermediate, rather than increase thermodynamic stability or prevent adsorption to surfaces. α-Crystallin thus appears to be an interesting additive for stabilising peptide and protein pharmaceuticals that are prone to fibril formation. However, further work is required to show that α-crystallin is tolerated upon injection and is stable throughout its intended life-cycle.

Poster No. III-21

SEQUENCE DETERMINANTS OF FIBRILLOGENESIS FOR HUMAN α–SYNUCLEIN

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The process of α–synuclein filament assembly is known to play a central molecular role in the pathogenesis of both idiopathic and familial forms of Parkinson's disease and dementia with Lewy bodies, as well as idiopathic multiple system atrophy and a number of other related neurodegenerative diseases in humans. These have been described as the α–synucleinopathies.
Our studies with recombinant α–synuclein, non-fibrillogenic β-synuclein and a large number of deletion, truncation and chimeric mutant forms of these two synucleins has allowed us to define sequence determinants for their fibrillogenic propensities *in vitro*.
Filament assembly was monitored by Thioflavin T fluorescence, far-UV circular dichroism and transmission electron microscopy.
The preliminary determinant was found to be a *global* measure of side-chain properties in terms of the mean total charge, the mean net charge, the mean β-strand propensity and the mean hydrophilicity of the protein. Thus the amino acid *composition*, rather than sequence seems to correlate with a thermodynamic propensity for nucleation. The subsequent determinant that also facilitates fibrillogenesis and in particular that appears to promote the formation of typical long filaments was found to be a local measure of β-strand propensity, namely 'β-strand contiguity'. This is calculated using a newly-developed algorithm ('SALSA') that employs a sliding window measurement of mean β-strand propensities of a wide range of peptide window lengths over the full length of the protein. We mutated the non-fibrillogenic protein β-synuclein to enhance either its global or local physicochemical properties in order to successfully produce β-synuclein proteins that readily assemble into filaments *in vitro*.

BENZON SYMPOSIUM No. 56

FUNCTIONAL AND PATHOGENIC PROTEIN AGGREGATION

AUGUST 24 – 27, 2009, COPENHAGEN, DENMARK

Organizing committee:

Daniel Otzen (Aarhus), Poul Henning Jensen (Aarhus), Sven Frøkjær (Copenhagen) & Niels Borregaard (Copenhagen)

Abstracts - THURSDAY, August 27, 2009

BACTERIAL AMYLOID BIOGENESIS IS COORDINATED BY DIRECTED NUCLEATION AND POLYMERIZATION

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Amyloid formation is a hallmark of many human diseases including Alzheimer's, Huntington's and the prion diseases. A new class of 'functional' amyloids provides a unique glimpse at how nature has harnessed the amyloid fiber to accomplish important physiological tasks. Organisms spanning much of cellular life can produce functional amyloids. We study the production of surface localized amyloids called curli, which are assembled by enteric bacteria. Curli fibers are associated with biofilm formation, host cell adhesion and invasion, and immune system activation. The major curli subunit protein, CsgA, polymerizes into amyloid after interacting the CsgB nucleator protein. CsgB presents an amyloid-like template to CsgA on the cell surface that initiates fiber formation. CsgA has five imperfect repeating units (R1-R5) that are each predicted to form strand-loop-strand structures. Asn and Gln residues in R1 and R5 were found to be required for efficient amyloid formation and for interaction with the CsgB nucleator protein. Furthermore, the polymerization of CsgA was tempered by the presence of conserved aspartic residues in R2, R3 and R4. When these aspartic acid residues were changed to alanine (CsgA*), polymerization was significantly faster *in vitro*. Even more remarkable was the observation that CsgA* assembled into an amyloid fiber *in vivo* in the absence of CsgB. The ability of CsgA* to polymerize into amyloid more efficiently, and in the absence of CsgB, was not without consequences. Cells expressing CsgA* grew more slowly when compared to cells expressing wild type CsgA. This analysis suggests that aspartic acid residues can potently inhibit functional amyloid formation. CsgA has apparently evolved to efficiently assemble into an amyloid *in vivo* only in the presence of CsgB. This suggests an elegant mechanism to control amyloid formation by regulating the temporal and spatial interactions between CsgA and CsgB.

NATURE AND PREVALENCE OF BACTERIAL AMYLOID

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Functional amyloids in bacteria have primarily been described in the

Enterobacteriaceae, such as *E. coli* where they are believed to be important in biofilm formation and for the physical structure of biofilms. Recently, by using in situ detection by Thioflavin T and conformationally specific antibodies of amyloids directly in biofilms, we found that functional amyloids are widespread among many phylogenetic groups (1) e.g. *Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Chloroflexi* and others in natural and engineered biofilms. More detailed investigations of various isolates and type strains have show a surprisingly high diversity of functional amyloids ranging from integration into the cell envelope to extracellular fibrils and spore coating. Especially among Gram positive bacteria a high diversity was found. Also well-known pathogens such as *Pseudomonas aerugenosa* express amyloidic fibrils and for P. *aerugenosa* fibrils we have extensively characterized these by biophysical methods, revealed the protein structure and identified the responsible genes. The findings suggest that the presence of functional amyloids so far has been almost completely overlooked in biofilms from technical, natural and human "ecosystems" where they may play multiple roles for the bacteria and the biofilm structure and function.

References:

1. Larsen, P., J.L. Nielsen, M. Dueholm, R. Wetzel, D. Otzen and P.H. Nielsen (2007): Amyloid adhesins are abundant in natural biofilms. *Environ. Microbiol.* 9: 3077-3090.

AMYLOID AND HAEMOSTASIS

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Misfolding is an inherent and potentially problematic property of proteins. Misfolded proteins tend to aggregate and the deposition of aggregated proteins, termed amyloid, is associated with a variety of highly debilitating diseases known as amyloidoses. A unique characteristic of misfolding in protein aggregation is the formation of crossbeta structure. Protein misfolding and aggregation is also increasingly recognized as the underlying cause of other health problems, including atherosclerosis and the immunogenicity of biopharmaceuticals. This raises the question how nature deals with the removal of obsolete proteins in order to avoid their accumulation and prevent disease. In recent years we have identified two homologous proteases, factor XII (FXII) and tissue-type plasminogen activator (tPA) that specifically recognize misfolded proteins. Factor XII is known as initiator of the contact system, an enzymatic cascade in blood that was known to become activated when blood contacts 'surface' materials, but its physiological activator remained elusive. Activated FXII was known to trigger two strikingly different events: (i) coagulation of blood by activation of factor XI and (ii) an inflammatory response through the generation of the vasoactive bradykinin via activation of prekallikrein into kallikrein. However, FXII seems not to be important for physiological hemostasis and its physiological role remained elusive. We found that FXII is activated by misfolded proteins with crossbeta structure and that this specifically leads to the formation of kallikrein and not to activated FXIa, both in vitro and in patients with systemic amyloidosis. These results identified misfolded proteins as activators of FXII and solved the paradoxical role of FXII in coagulation. tPA is known for its role in fibrinolysis, the process that results in the dissolution of blood clots. tPA is known to be activated by fibrin, the main component of a blood clot. We have demonstrated that tPA is activated by misfolded proteins with crossbeta structure in general. We also showed that fibrin has amyloid properties. Our discoveries have uncovered new

roles for the fibrinolytic system and the contact activation system beyond haemostasis.

MATERIAL PROPERTIES OF AMYLOID FIBRILS

Knowles TPJ[1], Oppenheim T[1], Buell AK[1], Meier C[1], White D[2], Dobson CM[2] & Welland ME[1]; [1]Nanoscience Centre, University of Cambridge, United Kingdom; [2]Department of Chemistry, University of Cambridge, United Kingdom E-mail: tpjk2@cam.ac.uk

The supra-molecular self-assembly of protein molecules into amyloid fibrils is a process with intricate connections with a range normal and aberant biological pathways. This talk focuses on the application of methods inspired by physics and nanotechnology to probe amyloid assembly as well as the characteristics and material properties of the structures formed as a result of fibrillar protein aggregation.

References:

1. T. P. Knowles, A. W. Fitzpatrick, S. Meehan, H. R. Mott, M. Vendruscolo, C. M. Dobson, M. E. Welland, Science, 21, 318 (2007)
2. T. P. J. Knowles, W. Shu, G. L. Devlin, S. Meehan, S. Auer, C. M. Dobson, M. E. Welland, PNAS 104 10016 (2007)
3. T. P. J. Knowles, J. F. Smith, A. Craig, C. M. Dobson, M. E. Welland, Phys Rev Lett 96, 238301 (2006)

PROTEIN ENGINEERING TO EMPLOY AMYLOID FIBRILS AS NEW MATERIALS

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Biological materials often exceed the characteristics and properties of man-made ones. One well-known example of fascinating natural materials are amyloid fibrils. However, employing amyloidogenic proteins for applications might encounter distinct risks. One of the simplest amyloid-like systems is reflected by silk proteins produced by arthropods. However, manufacturing silk proteins encountered many problems in the past. We developed a highly efficient bacterial expression system based on a gene engineering approach. The bacterial production system could be employed to mimic natural silk proteins as well as to engineer novel silk-like proteins. The basis is a new cloning strategy combining synthetic DNA modules and authentic gene sequences. The result is an industrial-scale fermentation of silk-like proteins with "engineered" properties. The recombinant silk proteins can be self-assembled into amyloid-like nanofibrils, hydrogels, spheres, and films. Additionally we have developed a spinning technique to produce spider threads closely resembling natural ones. The controlled design of silk proteins will generate novel bio-inspired materials for a variety of medical, pharmaceutical and technical applications.

DYNAMICS BEHAVIORS OF DESIGNER SELF-ASSEMBLING PEPTIDES

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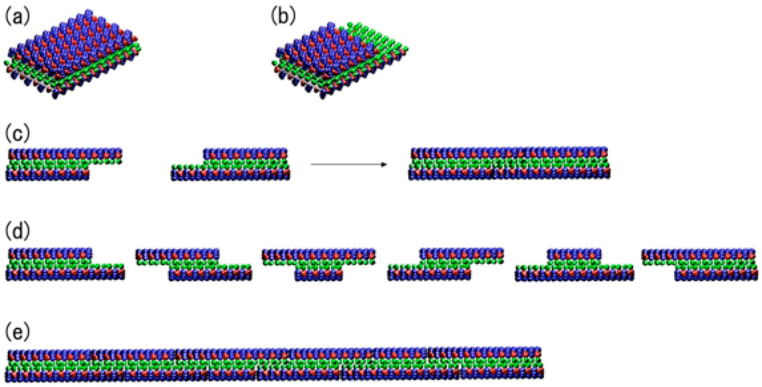
Self-assembly, disassembly and reassembly are a very important property for fabricating novel materials. In order to design the peptides as useful materials, it is necessary to fully understand the detailed processes and dynamics in order to design

better biological materials. In our systematic study using sonication, the mechanically breakdown short fragments of self-assembled nanofibers readily and repeatedly undergo reassembly. We proposed a plausible sliding diffusion model to interpret the reassembly involving complementary nanofiber cohesive ends. Unlike processed polymer fibers in which the fragments of polymers cannot readily undergo reassembly without addition of catalysts or through material processing, the self-assembly and reassembly is likely to be wide spread in many unrelated fibrous biological materials.

Our finding may have significant implication beyond supramolecular chemistry and biological materials self-assembly since nanofibers are also found in protein amyloid. It is possible that some of the self-assembled amyloid nanofibers will undergo reassembly to resist drug and other treatments.



Understanding dynamic amyloid nanofiber formation and finding a way to combat it still remains a formidable challenge.

References:

1. Yokoi, H., Kinoshita, T. & Zhang, S. (2005) Dynamic reassembly of peptide RADA16 nanofiber scaffold. *Proc. Natl. Acad. Sci. USA* **102,** 8414-8419.
2. Gelain, F., Bottai, D., Vescovi, A & Zhang, S. (2006) Designer self-assembling peptide nanofiber scaffolds for adult mouse neural stem cell 3-dimensional cultures. *PLoS ONE* **1, e119**.
3. Luo, Z., Zhao, X. Zhang, S. (2008) Structural Dynamic of a Self-Assembling Peptide d-EAK16 Made of Only D-Amino Acids. *PLoS ONE* 3, e2364.
4. Koutsopolous, S., Unsworth, L.D., Nagai, Y. & Zhang, S. (2009) Slow release of functional proteins through designer self-assembling peptide nanofiber hydrogel scaffolds. *Proc. Natl. Acad. Sci. USA* **106***,* 4623-4628.
5. Yang, Y. Khoe, U., Wang, X., Horii, A., Yokoi, H., & Zhang, S., (2009) Designer self-assembling peptides nanomaterials. *Nano Today* **4,** 193-210.

Poster No. IV-1

NOVEL CLASS OF FUNCTIONAL AMYLOIDS DISCOVERED IN PSEUDOMONAS

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It has been found that a unique type of fimbriae found among certain biofilm forming bacteria in the family *Enterobacteriaceae* shares many characteristics of the protein amyloids associated with neurodegenerative diseases. The properties of these fimbriae may provide insight into bacterial biofilm formation as well as amyloid build-up *in vivo*. This study presents the discovery of a novel functional amyloid expressed by a *Pseudomonas fluorescens* strain. The amyloid protein was purified and the amyloid-like structure verified by biophysical methods. Partial sequencing by MS/MS combined with full genomic sequencing of the *Pseudomonas fluorescens* strain allowed identification of the full gene which we term FapC. The fimbrin was found to contain a three time repeated motif with the consensus sequence $NNAX_4SX_2NX_2GNX_2GXNXAAGXGNQQXN$, where X is a non-aromatic residue. This

motif differed from those previously found in the curli fimbrins and prion proteins. The lack of aromatic residues in the repeat shows that aromatic side chains are not needed for efficient amyloid formation. The interactions of Asn and Gln residues seem to play a major role in the amyloid formation as these are highly conserved in curli, prion protein, and the newly identified fimbrin. The gene is conserved in many Pseudomonas strain including the opportunistic pathogen *P. aeruginosa* and is situated in a conserved gene cluster containing 6 genes of which one code for a homolog to FapC. This cluster may represent a new genetic organization for the production of functional bacterial amyloid.

Poster No. IV-2

DISCOVERY OF A LARGE REPERTOIRE OF PRIONS IN YEAST: A SYSTEM FOR HERITABLE PHENOTYPIC DIVERSITY

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Prions are proteins that convert between structurally and functionally distinct states, one or more of which is transmissible. In yeast, this ability allows them to act as non-Mendelian elements of phenotypic inheritance. To further our understanding of prion biology, we conducted a bioinformatic proteome-wide survey for prionogenic proteins in S. cerevisiae, followed by experimental investigations of 100 prion candidates[1]. Our studies of these naturally occurring glutamine/asparagine-rich (Q/N-rich) proteins provides evidence that many Q/N-rich domains have an inherent tendency to aggregate, yet that general sequence features, such as the ratio of asparagines versus glutamines, determine whether or not these aggregates are amyloid-like. In addition, we found that only amyloid-forming Q/N-rich sequences are capable of prion formation. The remarkable abundance of Q/N-rich regions in the yeast proteome is most likely the result of evolutionary forces that promote prionogenicity in important cell regulatory proteins to generate population-level heterogeneity. In accordance with this idea, we found that several candidate proteins can form genuine prions in yeast. These prions change the genotype-phenotype landscape in a heritable manner and create a major source of phenotypic variation that increases the adaptability of yeast populations to diverse environments.

References:

1. Alberti S, Halfmann R, King O, Kapila A, Lindquist S. (2009), Cell, *137.*

Poster No. IV-3

DEVELOPMENT AND INVESTIGATION OF NOVEL NANOMATERIALS BASED ON AMYLOID FIBRILS

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In addition to their implication in neurodegenerative diseases such as Alzheimer's and Parkinson's, a lesser known aspect of amyloid structures is their nature as high performance biomaterials.[1] These self-assembled and highly ordered nanostructures indeed possess interesting material properties. Their strength is comparable to that of steel and their mechanical stiffness is comparable to that of silk.[2] Nature has found ways to exploit these properties and amyloid fibrils in particular adopt important roles in biosynthetic pathways and as structural components in lower organisms.[3] Some proteins with propensities to form fibrils

are readily available, cheap and from sustainable resources. Their *ex vitro* fibrillization can be controlled by choosing appropriate denaturating conditions, thereby opening up possibilities in respect of scalability. However, the ability to fine-tune the functional properties of such amyloidic nanomaterials from natural proteins is a key issue on the way towards nanotechnological solutions.
In this contribution, we describe the hierarchical large scale self-assembly of amyloid fibrils and organic molecules into multicomponent or hybride biomaterials. In these higher order aggregates, the high aspect ratio of the amyloid fibrils is maintained and transferred to the macroscopic scale. In addition, we discuss the formation, structure and properties of these artificial macrofibers as well as the applicability of higher order aggregation as a general concept.
The mechanical stability of bare amyloid fibrils is mainly governed by their beta-sheet backbone, whereas their chemical properties are mainly defined by the functional groups of the peptide side chains. In this context, the chemical functionalization of the protein building blocks of non-artificial amyloidic nanostructures constitutes an amenable way to control their functional properties. We discuss the introduction of various functional groups into the fibrils and the effects of the modification on the chemical and mechanical properties. For instance, in contrast to the pristine fibrils the functionalized fibrils possess the ability to interact with other components and can be used to transfer nanoscopic ordering to the macroscopic scale.
References:
1. T. P. J. Knowles, A. W. Fitzpatrick, S. Meehan, H. R. Mott, M. Vendruscolo, C. M. Dobson and M.E. Welland. Role of intermolecular forces in defining material properties of protein nanofibrils. *Science* 318 (5858):1900-1903, 2007.
2. J. F. Smith, T. P. J. Knowles, C. M. Dobson, C. E. MacPhee and M. E. Welland. Characterization of the nanoscale properties of individual amyloid fibrils. *P. Natl. Acad. Sci. USA* 103 (43):15806-15811, 2006.
3. D. M. Fowler, A. V. Koulov, W. E. Balch, J. W. Kelly. Functional amyloid--from bacteria to humans. *Trends Biochem. Sci.* 32 (5):217-224, 2007.

Poster No. IV-4

AMYLOID-LIKE INTERACTIONS WITHIN NUCLEOPORIN FG-HYDROGELS

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Solid-state NMR spectroscopy (ssNMR) provides increasingly versatile techniques to probe protein aggregates and assemblies on different structural and motional levels [1]. We report progress in experimental design and hardware development that allowed us to study the structural organization and the kinetics of in vitro gelation of the 600 amino acid fxFG-repeat domain of the yeast nuclear pore protein Nsp1p. Nsp1p forms a hydrogel-based permeability barrier that excludes inert macromolecules, but allows rapid entry of nuclear transport receptors [2]. We find that the protein hydrogel is stabilized by mobile hydrophopic interactions as well as rigid inter-molecular β-sheets. The latter resemble N/Q-rich amyloids on the sequence and structure level. Our results have immediate implications for the possible functionality of the hydrogel as selective permeability barrier of the nuclear pore complex and our time-resolved ssNMR data provide insight into the consecutive steps of protein assembly during gel formation.
References:
1. Baldus M Journal of Biomolecular NMR (2007) 39, 73-86.
2. Frey S, Görlich D Cell (2007) 130, 512-523.

Poster No. IV-5

AMYLOID FIBRIL FORMATION BY THE *STREPTOCOCCUS MUTANS* ADHESIN P1

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Streptococcus mutans is the etiologic agent of dental caries. The Mr~185 kDa cell surface protein P1 (Antigen I/II, PAc) is a multi-functional adhesin that exists in a fibrillar layer on the cell surface and mediates interactions with salivary constituents, host matrix proteins, and other oral bacteria. The protein has repetitive discontinuous alanine- and proline-rich domains that interact to form complex conformational epitopes. The region intervening these sequences has been crystallized and is comprised of a â sandwich with 16 â strands arranged in 2 sheets, each containing 8 anti-parallel strands. Numerous sequences within P1 are predicted to promote amyloid formation based on computational algorithmic programs. Aggregates of native and recombinant P1 bound Congo red with higher absorbance and showed higher fluorescence with Thioflavin T (ThT) than non-aggregated protein samples. Aggregated native and recombinant proteins formed characteristic amyloid-like fibrils visualized by uranyl acetate staining and electron microscopy. A 10 amino acid sequence within the intervening region with a >98% prediction for beta aggregate formation using the TANGO program also formed fibrils, showed increased Congo red absorbance and bound ThT with higher fluorescence than the unaggregated peptide. A substituted peptide with a 0% beta aggregation prediction did not form fibrils, increase Congo red absorbance, or increase fluorescence of ThT. In frame deletion of the decapeptide from recombinant P1 abolished binding of monoclonal antibody 1-6F suggesting a perturbation of P1 structure and function. MAb 1-6F maps to the intervening region but does not bind to the peptide itself and is a strong inhibitor of *S. mutans* adherence to gp340, a component of the salivary pellicle and known physiologic ligand of P1. Our results suggest *S. mutans* P1 forms amyloid -like aggregates and this property contributes to its functional role as an adhesin.

(NIH DE08007)

Poster No. IV-6

NANOPARTICLE INFLUENCE ON AMYLOID PROTEIN AGGREGATION

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Amyloid formation follows similar routes regarding the nature of the protein involved. Many of the amyloidogenic proteins are related with diseases but also functional amyloids can be found in nature. Nanoparticles have been postulated for many medical applications such as drug delivery, imaging, etc… and nowadays their predominance on our society is increasing. It was recently shown that co-polymeric nanoparticles affect both fibrillation process of β2-microglobulin[1] and β-amyloid peptide.[2] This study has been extended to a wider range of particles and proteins to get a better understanding of the

interaction nanoparticle-protein. Particles with different surface area were used, and proteins, including pathogenic proteins as well as proteins not related to any disease but that nevertheless self aggregate into amyloid-like fibrils, were studied. The effect observed in a particular set of experimental conditions would depend on the balance between the different interactions, protein-protein and protein-nanoparticle. The addition of particles to the reaction media clearly affects the pre-equilibria involving the formation of oligomers. Depending on the strength and type of association on the particle surface depletion or concentration of protein monomers can occur, leading to catalysis or inhibition of the fibrillation process.

References:

1. Linse, S.; et al., Nucleation of protein fibrillation by nanoparticles. *PNAS*, **2007**, 104, (21), 8691.
2. Cabaleiro-Lago, C.et al., Inhibition of Amyloid beta Protein Fibrillation by Polymeric Nanoparticles. *JACS* **2008,** 130, (46), 15437

Poster No. IV-7

DECIPHERING PRPSC: CONFORMATION AND WHAT ELSE?

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Many lines of evidence support PrP^{Sc} as the only essential component of the prion and a protein conformational switch as the basis for its infectivity. PrP^{Sc} differs from its physiological form, PrP^{C}, in the C-terminal domain. This domain is globular and a-helix rich folded in PrP^{C} whereas it is largely aggregated and β-sheet rich in PrP^{Sc}. Once formed, PrP^{Sc} self-perpetuates by propagating as template its fold to PrP^{C}. In the absence of a pre-existing template that would assist the change, the involvement of a covalent modification, transient or irreversible and affecting either all or a minor subset of molecules, as a covalent trigger was postulated and never discarded. The combined use of protein chemistry and immunological tools has shown that sulfoxidation of M213 constitutes indeed a covalent signature for PrP^{Sc}. This modification was identified in some but not all the PrP chains from the PrP^{Sc} of given specie and it was present in the PrP^{Sc} of all the species tested. A molecular dynamics study of the effect of this modification has shown that sulfoxidation of any of the Helix-3 methionines impacts on the stability of the α-fold increasing the flexibility of preceding regions, perturbing the network of stabilizing interactions and favoring the productive pathway of the pathogenic conversion. Such predicted structural destabilization can be probed using oxidized-like and oxidation-prone mutant chains of the α-fold. All these results suggests a crucial role for the imbalance of oxidative insults repairment, as those described on ageing, at the prion birth.

Poster No. IV-8

SURFACE INDUCED FIBRILLATION OF INSULIN

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In many pharmaceutical systems interfaces and surfaces are presents, e.g., the air-water interface present in liquid formulations or the interfaces created during the preparation of drug delivery systems or the solid interface in the device. The

question is if these surfaces can induce structural changes, aggregation and potentially fibrillation, but also how pharmaceutical development can improve the potential stability issue of the protein drug.
The present study elaborates on the effects of adsorption to solid surfaces on the physical stability, structure, and fibrillation of insulin. The model proteins used are insulin as well as analogues thereof, as insulin is one of the more studied proteins and much basic information as such is know already. Three different insulins are used in the present experiments; they range from human insulin, containing two Zn2+ pr hexamer, to the modified insulin's: zinc-free insulin and AspB28 insulin. The potential in using these analogues is essentially to vary the distribution between monomer, dimer, and hexamer, and thereby the influence of this distribution on the tendencies to fibrillate. The Teflon-particles used as model for solid surfaces are stable in solution without any surfactants present, and they have a refractive index close to water, both of which properties that make them unique in the studies of surface adsorption to solid surfaces.

Poster No. IV-9

A SEQUENCE IN *CANDIDA ALBICANS* ALS5P MEDIATES AMYLOID FORMATION AND CELL AGGREGATION

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The yeast *Candida albicans* can cause severe human infections. The cell wall-bound Als adhesins have been implicated in the pathogenicity of *C. albicans*. One of the critical early steps of infection is the ability of the cells to aggregate, which can lead to the formation of therapeutically resistant biofilms. The adhesin Als5p consists of an N terminal signal sequence, three tandem Ig-like domains, a conserved threonine rich (T) domain, 36-mer tandem repeats, a glycosylated stalk region, and a GPI anchor. We have shown that the *C. albicans* Als5p can form amyloid fibers which we hypothesize are critical for aggregation. The TANGO β-aggregate predictor identified a potential amyloid forming sequence in the T domain of Als5p. Synthetic peptides corresponding to residues 322-334 in this region showed amyloid formation by congo red absorbance, thioflavin T fluorescence, and fiber formation (Otoo et al., Euk. Cell 7 (5): 776-782, 2008). TANGO also predicted that a V326N mutation would have greatly decreased potential to form amyloids. This prediction was confirmed with synthetic peptides: a peptide containing the native sequence of residues 322-334 formed amyloids whereas a peptide with a V326N mutation did not. To test our hypothesis that Als5p amyloid fibers are critical for cell aggregation, we generated Als5p and Als5p^{V326N} constructs expressed in a *S. cerevisiae* surface display model. Expression of full-length Als5p wild type amyloid-forming protein mediated cell aggregation. On the contrary, expression of the Als5p^{V326N} mutant did not cause cells to aggregate, demonstrating that the single amino acid change in the T domain prevented amyloid formation and cell aggregation. These data suggest that the conserved T region sequence mediates amyloid fiber formation that is critical for yeast cell aggregation.
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Poster No. IV-10

MOLECULAR DISSECTION OF THE PRION-FORMING DOMAIN ON THE YEAST SUP35P PROTEIN

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The study of yeast prions has provided important information that is helping us to better understand the mechanisms that control prion protein aggregation and cell-to-cell transmission of prion seeds (propagons). We are using [*PSI*$^+$], the prion form of the yeast protein Sup35p (eRF3) as our model. Sup35p is an essential translation termination factor and when is in its prion form this leads to an impairment of translation termination and detectable stop codon readthrough. The N terminal region (residues 1-123), of Sup35p constitutes the region essential for prion formation (prion-forming domain; PrD). The PrD consists of two distinct regions: a Gln/Asn-rich region (QNR; residues 1-40) and an adjacent region containing five copies of an oligopeptide repeat (OPR; residues 41-97). Various single amino acid substitutions in Gly-Gly pairs in the second oligopeptide repeat impair or completely block the propagation of the [*PSI*$^+$] prion as does deletion of this repeat and in some cases act as dominant negative propagation mutants. Deletion of a sequence between repeat 1 and repeat 2 also impair propagation indicating that the repeats per se are not sufficient for propagation. NMR studies suggest that propagation-defective forms of Sup35p have constrained conformational flexibility in the PrD leading to a failure to produce sufficient numbers of propagons to sustain propagation in dividing [*PSI*$^+$] cells.

Poster No. IV-11

***p*-CYANOPHENYLALANINE: AN INTRINSIC, SITE-SPECIFIC PROBE OF AMYLOID FORMATION**

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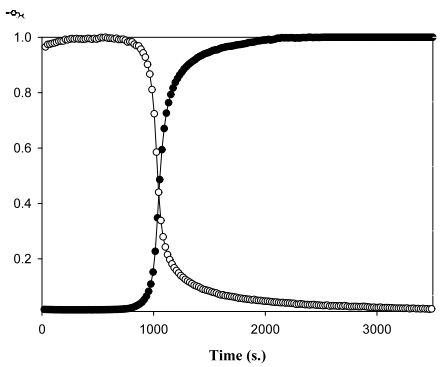
Amyloid formation is traditionally followed using global probes, which cannot provide residue specific information. Here we demonstrate that *p*-cyanophenylalanine (F_{CN}) provides a non-invasive, site-specific probe of amyloid formation using islet amyloid polypeptide (IAPP) as an example. IAPP is a 37 residue peptide co-stored and co-secreted with insulin from the β-cells of the pancreas. IAPP readily forms amyloid *in vitro* as well as *in vivo* during type II diabetes. Three variants of IAPP were made in which the three aromatic residues were each replaced with F_{CN}. The fluorescence of F_{CN} is considerably higher in water than when it is buried in a hydrophobic environment, allowing the local environment of the side chain to be probed.



Comparison of the fluorescence of thioflavin-T (●) and F_{CN} (○) measuring the time course of fibril formation for hIAPP Y37F_{CN}. The molecular representation of F_{CN} is shown as an inset.

Each variant of IAPP formed amyloid fibrils that appear identical to those formed by wild type, as judged by CD and transmission electron microscopy. The time course of the fibrilization reactions for each variant was monitored by both thioflavin-T and F_{CN} fluorescence, which were essentially identical

for each variant, indicating that the residues at the 15, 23 and 37 positions become buried concomitantly with fibril formation. Fluorescence quenching studies allow the solvent accessibility of each F_{CN} site to be probed during the lag and fibril phases of amyloid formation. F_{CN} analogs of IAPP allow the testing of putative inhibitors, which give false positives in thioflavin-T assays, as illustrated by our recent work on rifampicin.

Poster No. IV-12

PURIFICATION OF AMYLOIDS FROM BACTERIA

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Amyloid fibrils have historically primarily been associated with degenerative diseases including Alzheimer's and Parkinson's diseases. Interestingly, studies of many microorganisms show that amyloid fibril formation also provides biological functions. Such functional bacterial amyloids (FUBA) have been identified and characterized on surfaces of bacteria such as *E. coli,* salmonella species and certain actinomycetes. However, studies of natural biofilm using conformationally specific antibodies and Thioflavin T staining have revealed that many other, so far unknown bacteria also produce FUBA indicating existence of a large diversity of amyloid structure and functions [1]. The aim of this project is to reveal some of this diversity by purification of a range of bacterial amyloids and detailed structural and functional analysis. Surface associated amyloids were examined on isolates from different sources and various strains from culture collections by conformationally specific antibodies and Thioflavin T staining. Purification of amyloid is a challenge due to its insoluble behavior so a range of methods was used for FUBA purification including freeze-thaw cycles, treatment with enzymes, SDS and preparative SDS-PAGE. It was clear that no universal method could be applied for all isolates tested and an optimization was needed for each species. The purified amyloids were examined and verified by using array of biophysical, morphological and histochemical analysis: Fourier Transform infrared spectroscopy, Circular Dichroism, Transmission Electron Microscopy, and Thioflavin T staining. It is anticipated that the detailed knowledge about a diverse range of amyloid will enhance the understanding of the role of amino acid composition on structure and function of FUBA.

References:

1. Larsen, P., J.L. Nielsen, M. Dueholm, R. Wetzel, D. Otzen and P.H. Nielsen (2007): Amyloid adhesins are abundant in natural biofilms. *Environ. Microbiol.* 9: 3077-3090.

Poster No. IV-13

A BIOSENSOR ASSAY FOR AMYLOID GROWTH IN CROWDED ENVIRONMENTS

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Macromolecular crowding has been shown to modulate the kinetics and thermodynamics of protein folding and aggregation through excluded volume or depletion interactions, limitation of diffusive transport and compaction of the polypeptide chain. Such contributions to protein aggregation become increasingly relevant when attempts are made to relate hypotheses from *in vitro* studies to *in*

vivo observations and may also play a major role in the fabrication of materials based on amyloid structures. A label-free biosensor technique based on the quartz crystal microbalance was adopted to directly monitor amyloid fibril elongation in crowded environments and is demonstrated here to be a facile assay for systematically probing the effect of crowding on the aggregation pathway of several proteins. This biosensor based approach has identified the differential actions of macromolecular crowding on protein aggregation and has shown that the dominant effect is highly dependent on the macromolecule used to simulate crowding and on the protein under investigation. Specifically, an effect of cosolute size was observed together with a difference in the aggregation kinetics of structured versus disordered polypeptides under the same crowded conditions.

Poster No. IV-14

RECOGNITION OF INTERMEDIATE SPECIES IN ALPHA–SYNUCLEIN SELF-ASSEMBLY USING ESIPT DUAL EMISSION PROBES

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Self-organisation of the natively unstructured monomeric presynaptic 140 aa protein alphα-synuclein (AS) is often associated with Parkinson's disease, characterized by loss of dopaminergic neurons in the midbrain. It is well described that aggregation of AS leads to the highly organized fibrillar aggregates with a characteristic cross-beta amyloid structure. However, relatively little is known about the detailed mechanism of aggregation, primarily due to the low concentration and stability of intermediate structures and a lack of suitable methods for their identification. At the same time there are many indications that cytoxicity arises from the intermediate species formed during AS assembly and may involve pathological pore-formation in cellular or organelle membranes.

We have developed fluorescent dyes that are able to sense intermediate self-organized assemblies of AS that are forming before the classical fibrils. These dyes exhibit a dual fluorescence due to the Excited State Intramolecular Proton Transfer (ESIPT) reaction. Their blue shifted band of fluorescence arising from the normal excited species (N*) and a red shifted band is due to the excited state tautomer (T*). The ratio between the bands is a measure of the difference in the microenvironment of the dye. It thus provides a good indication of the folding processes occurring through the self assembly pathway. Current attempts are directed at the use of such probes for the identification and isolation of the intermediate species. We will present the structure and morphology of some of these self organized assemblies of AS, information gained from the combined use of numerous techniques.

Poster No. IV-15

Methionine oxidation induces amyloid fibril formation by full length apolipoprotien A-I

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Apolipoprotein A-I (apoA-I) is the major protein component of HDL, and functions as a cholesterol transporter. The deposition of apoA-I derived amyloid deposits is associated with various hereditary systemic amyloidoses and atherosclerosis; however, very little is known about the mechanism of apoA-I amyloid fibril formation. Methionine residues in apoA-I are oxidised *via* several mechanisms *in vivo* to form methionine sulfoxide [met(O)], and significant levels of methionine oxidised apoA-I [met(O)apoA-I] have been detected in human serum. We investigated the effect of methionine oxidation on the structure, stability, and aggregation of full length, lipid free apoA-I. Circular dichrosim spectroscopy showed that oxidation of all three methionine residues in apoA-I caused partial unfolding of the protein and decreased its thermal stability, reducing the melting temperature (T_m) from 58.7±0.2 °C for the unoxidised protein to 48.2±0.4 °C for met(O)apoA-I. Analytical ultracentrifugation revealed that methionine oxidation inhibited the natural self association of the protein to form dimers and tetramers. Incubation of met(O)apoA-I for extended periods at pH 6.0 and 37 °C resulted in aggregation and precipitation of the protein, and this was found to be concomitant with an increase in thioflavin T fluorescence in the sample. Inspection of the aggregates by electron microscopy revealed apparently flexible, fibrillar structures with a ribbon-like morphology and a width of ~11 nm and lengths of up to several microns. X-ray diffraction studies of these fibrils in a semi-dehydrated state revealed a scattering pattern with peaks at 4.7 Å and 10.1 Å consistent with a cross-beta amyloid structure. This study is the first systematic report of fibril formation by full-length, wild-type apoA-I and represents the first demonstration that methionine oxidation can induce amyloid fibril formation.

Poster No. IV-16

Bacterial Macroscopic Amyloid-like Fibers with Cytotoxic and Adhesive Properties

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We present a body of ultrastructural, biochemical, and genetic evidence that demonstrates the oligomerization of virulence-associated autotransporter proteins EspC or EspP produced by deadly human pathogens enterohemorrhagic (EHEC) and enteropathogenic (EPEC) *Escherichia coli* into novel macroscopic amyloidogenic rope-like structures. These proteins exhibit serine protease and cytotoxic activities on host cells and are self-exported to the milieu via a type 5 secretion mechanism. The rope-like structures showed characteristics consistent with human amyloidogenic proteins including, high aggregation and insolubility, stability to anionic detergents and high temperature, a β-spine structure, and Congo Red binding. These macroscopic ropes were not observed in cultures of non-pathogenic *E. coli* or isogenic *espP* or *espC* deletion mutants of EHEC or EPEC but were produced by an *E. coli* K-12 strain carrying a plasmid expressing *espP*. The ropes bound to and destroyed dramatically cultured epithelial cells, served as a substratum for bacterial adherence and biofilm

formation, and protected bacteria from antimicrobial compounds. We hypothesize that these ropes play a biologically significant role in the survival and pathogenic scheme of these organisms.

Poster No. IV-17

Hydrogen Exchange Studies of a HET-s Homologue Capable of Species Barrier Breaching

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FgHET-s, a homologue of the HET-s protein prion of *Podospora anserina,* has been identified in the fungus *Fusarium graminearum*, a major pathogen of wheat and other cereals. Amyloid fibrils formed by the prion forming domain of HET-s (HET-s(218-289)) have been extensively characterized by quenched hydrogen exchange detected by NMR [1], and by solid state NMR [1,2]. FgHET-s(218-289) and HET-s(218-289) display only 36% identity. FgHET-s forms amyloid fibrils *in vitro* and a FgHET-s(218-289)-GFP fusion protein is able to propagate as a prion *in vivo* in *P. anserina*. *In vitro,* FgHET-s(218-289) can seed HET-s(218-289) fibrillisation (and vice versa). *In vivo*, the *F. graminearum* [FgHet-s] prion induces *P. anserina* [Het-s] formation. These results indicate a species barrier breaching between the *P. anserina* and *F. graminearum* prions. Hydrogen/deuterium exchange detected by NMR and solid state NMR data show that the fold and a number of structural details are identical for the prion-forming domains of the two proteins. This structural similarity readily explains the species barrier breaching phenomenon that occurs here in spite of extensive sequence divergence.

References:

1. Ritter C, Maddelein ML, Siemer AB, Lührs T, Ernst M, Meier B, Saupe S, Riek R (2005). Correlation of structural elements and infectivity of the HET-s prion. Nature *435(7043),* 844-848.
2. Wasmer C , Lange A, Van Melckebeke H, Siemer AB, Riek R, Meier BH (2008). Amyloid fibrils of the HET-s(218-289) prion form a beta solenoid with a triangular hydrophobic core. Sciene 319(5869):1523-6.