

We suggest that antiparallel β -sheet structure might represent a distinctive signature of amyloid oligomers (Cerf et al, *Biochem J*, 2009, 421:415-23) underlying their common pathogenic action.

2236-Pos Board B6

Amyloid Beta Peptide: The Influence of Intrinsic Factors on Fibril Formation

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Alzheimer's disease (AD) is the most common form of dementia. Studying the amyloid β -peptide (A β) involved in the pathogenesis and understanding the mechanism of the disease will hopefully lead to developing drug candidates that could cure or delay the onset of AD.

One way of approaching this is to inhibit the fibril formation. Mutating the peptide gives an insight on which amino acids are important in the aggregation mechanism. We have investigated the importance of the aromatic rings in the phenylalanine side chains in position 19 and 20 by substituting these with leucine. This way the hydrophobicity is preserved while aromatic stacking possibilities are removed. Single and double mutants were expressed in *E. coli* from synthetic genes. The kinetics of fibril formation was followed using a highly reproducible thioflavin T assay and show that A β (M1-42)F19L aggregates slower than the wild type, mainly observed as a longer lag time. Also the structural change, monitored by far-UV circular dichroism spectroscopy, differs between wild type and all mutants. This indicates that the phenylalanine rings have a role in the amyloid formation. With cryo-TEM it was confirmed that all peptides form fibrils with similar morphology.

The influence of the slower F19L mutant was further studied in co-aggregation experiments together with the wild type. Preliminary results show that the lag time of the mixture ends up in between those of the wild type and the F19L mutant, and that the lag time depends on the ratio of the two peptides as well as the total concentration. Further co-aggregation studies include a familial mutant peptide with faster aggregation kinetics than the wild type, as well as seeding experiments in which wild type is seeded with fibrils from F19L or the familial mutant.

1(1)

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The Effect of a Membrane Mimicking Detergent on Alzheimer's Amyloid Peptide Aggregation Studied by EPR

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The aggregation of the β -Amyloid (A β) peptide into fibrils is the chief indicator of Alzheimer's disease. However, it seems that small, oligomeric aggregates, rather than fully formed fibrils could be the toxic species. A β -membrane interaction could also have an effect, so here effect of the membrane-mimicking detergent SDS is studied.

We measure, by spin-label-mobility EPR, A β aggregation at different SDS concentrations. It monitors all peptide in the sample and is not limited to particular aggregate sizes. The high-SDS form found (C) is consistent with the one-peptide/micelle model [1-2]. In the absence of SDS (A) the high concentration of A β (0.55 mM) most certainly enforces fibrils. At intermediate SDS (B), EPR reveals an increase in particle size suggestive of oligomer formation, eventually involving detergents. Thus a first glimpse into the behavior of SDS at sub-CMC (Critical Micelle Concentration) SDS concentrations, where high resolution techniques, such as NMR, fail because of particle size limitation, [1-2] are accessible.

[1] FEBS, Wahlström et al. (2008) 275: 5117-5128.

[2] J. Biomol NMR, Jüri et al. (2007) 39: 63-72.

2238-Pos Board B8

A Conventional and 2DCOS Infrared Study of Amyloid Fibril Formation

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Amyloidosis is characterized by the abnormal self-assembly and deposition of proteinaceous material into insoluble ordered aggregates. There are several

pathologies associated with this aggregates, known as "protein misfolding" disorders. In many proteins, when heated at high temperature and low pH, a series of structural changes resulting in the formation of fibrillar structures are produced. Insulin is a model of fibril formation that has produced a wealth of biochemical and structural data. The time-course of fibril formation can be followed by infrared spectroscopy looking at the appearance of a characteristic band in the amide I region. The kinetics is triggered by temperature at 70 °C and pH at 2.3. The infrared spectrum shows, that after a lag time (concentration-dependent), α -helix and β -turns bands decrease first whereas the random coil component increases. Later, a band at 1626 cm⁻¹, associated with extended chains, replaces the random coil component. Infrared 2D-COS has been applied to different stages of the process. Maps have been formed at different incubation times: before random coil formation and at different stages in the random coil-fibril exchange. The synchronous maps do not give in principle much information because they are dominated by the appearance of the band due to the fibril. In order to extract such information we have developed an approach in which we take a window and move it along the perturbation (2D-moving lapse). This, together with the asynchronous maps indicate a two-step process with a first stage associated with an opening of the protein driven by destabilization of β -turns, located in the outer part of the protein, and a second part where the fibril is formed.

This work was supported in part by the Spanish Ministerio de Educación y Ciencia (grant No. BFU 2010-22103).

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Mechanism of Non-Specific Inhibitors of Amyloid Assembly: Interactions of Lacmoid with the Amyloid Beta Peptide

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Increasing evidence shows a strong link between the self-assembly of the amyloid beta peptide (A β) and the pathogenesis of Alzheimer's disease. Soluble oligomeric A β assemblies are thought to be the toxic species causing synaptic and neuronal injury in the patient's brain. Many inhibitors for the oligomerization and/or fibrillation process of neurodegenerative diseases have been reported, yet only little is known about the mechanistic details of these compounds. The present studies concern the interaction of one such inhibitor, lacmoid. We investigated this interaction by a broad biophysical approach revealing similar binding characteristics to A β as has been reported for detergents. Furthermore, we show that lacmoid has the ability to inhibit both oligomeric assembly and fibrillation of A β . Nuclear magnetic resonance experiments show an overall signal decrease upon addition of lacmoid while the chemical shifts only display small changes. High lacmoid concentration causes a loss of the major part of NMR signals including ¹H-¹⁵N-HSQC, ¹H-¹⁵N-TROSY and ¹H-¹³C-HSQC cross-peaks. Circular Dichroism spectroscopy was applied to monitor the kinetic aggregation process of A β in presence of lacmoid. Low lacmoid concentrations slow down the conversion from a random coil-like towards a beta-sheet state while at high concentration lacmoid completely prohibits secondary structure changes.

Taken together, these findings provide the basis for a simple model which could explain how non-specific interactions with small molecules interfere with amyloid formation. Understanding the mechanistic details is potentially helpful for future drug design of small molecule therapeutics targeting amyloid disorders, such as Alzheimer's disease.

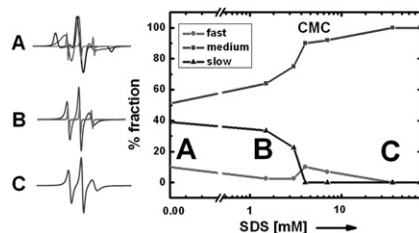
2240-Pos Board B10

Understanding Formation and Structure of Peptide Nanofibers via Steered MD Simulations

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Understanding the formation and structure of fibers formed via peptide aggregation is a major challenge for both prevention of diseases and also for utilising such structures for nanostructure templating. Here, we propose that single molecule pulling experiments can be used to characterise the mechanical and structural properties of these fibers. We demonstrate this idea via molecular dynamics simulations on fibers formed by a library of triblock peptides. In these oligomers, hydrophobic residues in the central block are replaced to control the resulting fibers. Our results suggest that, independent of the hydrophobic residue type, all these peptides yield an inter-dimer spacing of ~ 0.48 nm along the fiber axis. Hence, backbone hydrogen bonding alone dominates and the hydrophobic residues simply squeeze into the hydrophobic core of these fibers. We measure the relative strength of these fibers via steered MD simulations. Our results suggest that hydrophobic residues such as tyrosine and tryptophan not



only bring stability to the fibers, but also determine the specific orientation of individual peptides within these nanofibers.

2241-Pos Board B11

Second Virial Coefficients as Determined using Self Interaction Chromatography and Protein Aggregation in Solution

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One of the key parameters for describing protein self-interactions in solution is the second virial coefficient; B_{22} . Measurement of the second virial coefficient is important for describing the solution behaviour of proteins, especially as it relates to precipitation, aggregation and crystallisation phenomena. Due to its quick analysis time and use of small amounts of protein, Self Interaction Chromatography (SIC) is becoming an increasing popular approach. This paper includes a detailed description of the best experimental practise for running SIC experiments on aqueous protein solutions.

SIC data is presented here for a series of protein systems studied including lysozyme and lactoferrin, for a wide range of salts and their concentrations. The aggregation kinetics for these two proteins have also been obtained using Dynamic Light Scattering (DLS) for the same solution conditions. Remarkably, SIC confirmed the repulsive nature of B_{22} values of lactoferrin for all salts and concentrations tested. This data agreed completely with DLS data which confirmed the exception stability of lactoferrin to aggregation in solution. In contrast, aggregation behaviour of lysozyme was a strong function of salt species present as well as their concentrations. This aggregation behaviour correlated well with B_{22} values obtained by SIC. This work confirming the value of SIC as a useful technique for predicting the aggregation behavior of proteins in solution via B_{22} data.

2242-Pos Board B12

The Role of Interfaces in the Nucleation of Amyloid Nanotubes

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The nucleation and growth pathways of cross- β peptide aggregates are widely studied but not fully understood. Using Amyloid- β (16-22), the nucleating core of the Amyloid- β protein, and a rhodamine 110 -labeled peptide (Rh1722) that co-assembles with Amyloid- β (16-22) nanotubes without changing morphology, we investigate the role of interfaces in amyloid nucleation and self assembly. The high sensitivity of the Rhodamine 110 lifetime to its local environment provides a metric for structural heterogeneity, which is exploited in these studies using Fluorescence Lifetime Imaging Microscopy (FLIM). Specifically, we identify the importance of air-solvent and solvent-glass interfaces in the nucleation of cross- β peptide nanostructures and apply FLIM to demonstrate that different interfaces can lead to distinct nucleation and self-assembly pathways and also to differences in the structure of assembled peptides.

2243-Pos Board B13

Single Molecule Studies of Interaction Between Alzheimer's Amyloid- β Peptides of different Lengths

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The oligomers formed by amyloid- β ($A\beta$) peptides are thought to play a causative role in Alzheimer's disease, possibly due to membrane permeabilization by small $A\beta$ oligomers. Two primary variants of $A\beta$, of lengths 40 ($A\beta_{40}$) and 42 ($A\beta_{42}$) amino acids are produced at defined ratios in a normal individual. Changes in the ratio of $A\beta_{40}/A\beta_{42}$ have been shown to correlate strongly with the disease. Further, a mixture of the two forms of the peptide has been shown to exhibit different fibrillization kinetics in vitro and to elicit different extents of cellular toxicity. Thus, mixed $A\beta_{40}/A\beta_{42}$ oligomers can have different characteristics compared to oligomers formed by either one of the peptides. However, most research in the field focuses on one of the peptides at a time. Further, the $A\beta$ oligomers are heterogeneous, metastable and physiologically occur at low nanomolar concentrations, which makes it difficult for the use of conventional techniques to identify the toxic oligomers. In our work, we use single molecule methods to overcome these obstacles and study the formation and the evolution kinetics of mixed oligomers of $A\beta_{40}$ and $A\beta_{42}$ at different ratios in solution. We employ single molecule FRET and photobleaching of two different fluorophores attached to the N-terminal of $A\beta_{40}$ and $A\beta_{42}$ to determine the oligomer size and composition. Extending these studies to oligomer formation and binding kinetics of the two peptides on model membranes constructed from brain lipid extracts will provide a critical new understanding of how the stoichiometry of interaction of the two peptides both in solution and on the membrane surface affects the composition and permeabilizing potency of the resulting mixed oligomers.

2244-Pos Board B14

Structures and Dynamics in Amyloid-Beta Dimers: Effects of Zinc Binding and Chelation

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Alzheimer's disease (AD) is causally linked to the self-association of amyloid- β peptide ($A\beta$), a small protein of 39-43 amino acids. Historically, $A\beta$ fibrils found in extracellular senile plaques were thought to be the pathogenic agents; however, recent evidence suggests that $A\beta$ oligomers as small as dimers are more closely linked to Alzheimer's symptoms and progression. We have employed Förster resonance energy transfer (FRET) measurements to probe structures and dynamics in dimers of $A\beta_{40}$. Results for single, surface-tethered dimers reveal two characteristic FRET efficiencies, perhaps indicative of two preferred dimer structures; these values are confirmed by fluorescence lifetime measurements in bulk solution. Here, we report comparative studies in the absence and presence of zinc, which is thought to promote $A\beta$ oligomer formation at synapses. The effects of the zinc chelator clioquinol in reversing zinc-induced structural change will also be discussed.

2245-Pos Board B15

Comparison of Epitaxially and Solution-Grown Amyloid β_{25-35} Fibrils

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Amyloid fibrils play a key role in a wide range of protein misfolding disorders. Amyloid β peptides form self-associating fibrillar structures in Alzheimer's disease. The biologically active, toxic fragment of the full-length beta peptide, the $A\beta_{25-35}$ peptide is able to form an oriented network on mica by an epitaxial assembly mechanism. Whether the structure of the epitaxially grown fibrils is similar or identical to that of the fibrils assembled under equilibrium conditions is not known. To explore these differences, we investigated fibril structure and dynamics with atomic force microscopy, force spectroscopy (AFM) and Fourier transform infrared (FTIR) spectroscopy methods.

According to our AFM experiments the epitaxially-grown fibrils were significantly different from solution-grown fibrils in terms of their morphology and formation kinetics. The fibril height was 1-3 nm for the epitaxially-grown fibrils, whereas solution-grown fibrils were 7-40 nm thick. Unlike epitaxially-grown fibrils, fibrils assembled in solution displayed a presumably helical structure. While fibril assembly in solution occurred on a time scale of hours to days, on mica surface fibrils appeared within a few minutes. The nanomechanical behavior of $A\beta_{25-35}$ fibrils was characterized by the appearance of force staircases which correspond to the force-driven unzipping and dissociation of protofilaments. Both types of fibrils showed similar plateau-like nanomechanical responses, however the plateau-force distribution was unimodal for epitaxially-grown fibrils and bimodal for solution-grown fibrils. The IR spectra contained an intense peak indicative of beta-sheet structure: 1630 cm^{-1} and 1623 cm^{-1} for epitaxially grown fibrils and for fibrils assembled in solution respectively. The shift in the amide I band towards smaller wavenumbers indicates a more compact structure. Thus, while both fibrils types display an underlying beta-sheet structure, they are slightly different: solution-grown fibrils are more compact with a pronounced axial periodicity.

2246-Pos Board B16

Cellular Internalization of Monomeric and Oligomeric Amyloid-Beta 42 Peptide

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Amyloid beta ($A\beta$) is the major component of extracellular plaques found in Alzheimer's disease (AD). Uptake of $A\beta$ from extracellular to intracellular space, which is likely by endocytosis, appears to be an important process for understanding AD pathology (Friedrich et al., PNAS 2010; Hu et al., PNAS 2009). It may also be a promising target for treatment and prevention.

We aimed to study biophysical details of $A\beta$ uptake, and to determine the uptake kinetics for different $A\beta$ forms: monomers, oligomers, higher molecular mass aggregates of $A\beta$ in and small fibrils. We used neuroblastoma (Sh-EP) cells as a model for neurons. Uptake kinetic were followed by using the fluorescent labelled $A\beta_{42}$ that was monomerized and was purified by gel filtration. $A\beta_{42}$ peptide was added to the cell culture medium and the amount of intracellular aggregates was quantified using automated fluorescence microscopy.