View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector



**1226-Plat**

**Deciphering the β-Structure Interconversion of the Alzheimer's Amyloid-β Peptide Along Fibrils Formation**

Rabia Sarroukh, Régis Gasper, Emilie Cerf, **Jean-Marie Ruysschaert**, Erik Goormaghtigh, Vincent Raussens.
Université Libre de Bruxelles, Brussels, Belgium.

Alzheimer's disease (AD) is the most common progressive and fatal neurologic condition. One hallmark of this disorder is characterized by polymorphous extracellular deposits called ''senile plaques''. Amyloid- beta peptide (Aβ) is the primary component of these plaques and plays an importani, but not completely understood, role in the neurotoxicity. Aβ forms different entities: monomeric, large soluble entities collectively called oligomers and insoluble fibrils. The mechanisms leading to accumulation of misfolded peptide and the fibrils formation remain a matter of debate.

Two-dimensional infrared correlation analysis of Aβ1-42 was used to monitor the nature of structural transitions along the aggregation process. First, concerted conversions head to a decrease in helical and/or random coil structure in favour of β-sheet content. Once β-sheet appeared, we found that Aβ1-42 peptide adopted in a first stage an antiparallel β-sheet structure assigned to toxic oligomers. The final state of aggregation displaying parallel β-sheet structure as expected for fibrils. On IR spectra an evolution of the main band absorbance of β-sheet structure in the Amide I spectral region (1700-1600 $cm^{-1}$) to higher wavenumbers is observed. We assign this bandshift using 2-D correlation analysis to the interconversion of oligomers into fibrils by a reorientation of β-strands (probably by a 90° rotation) along their axes, allowing the transition from antiparallel to parallel β-sheet.

This work addresses new point in antiparallel β-sheet structure implications in aggregation as well as in the kinetic of fibrils formation which were so far contentious. This structure seems particularly important for transient toxic intermediates and required for further aggregation.

[1] E. Cerf, R. Sarroukh et al. Antiparallel beta-sheet: a signature structure of the oligomeric amyloid beta-peptide, Biochem.J. 421 (2009) 415-23.

**1227-Plat**

**Effects of Heparin on Amylin Fibrillization**

**Andrei T. Alexandrescu**, Suman Jha, Sharadrao M. Patil, Jason Gibson, Nathan N. Alder, Craig E. Nelson.
University of Connecticut, Storrs, CT, USA.

Amylin is an endocrine hormone that helps regulate blood glucose levels and controls appetite. In patients with type 2 diabetes, amylin misfolds into amyloid plaques that have been implicated in the destruction of the pancreatic β-cells that synthesize insulin and amylin. The plaques found *in situ* contain not only amylin but heparan sulfate proteoglycans that attach to the fibrils through their glycosaminoglycan chains. We investigated the interaction of amylin with heparin fragments of defined length, that model the glycosaminoglycan chains associated with amyloid deposits. We found that heparin enhances fibrillization in a manner that depends on the length of the polysaccharide fragments. We used NMR to establish that the negatively charged heparin fragments bind to the positively charged N-terminal half of amylin. We used FRET to determine that heparin associates with amylin fibrils rather than enhancing fibrillization catalytically. We used TIRFM to show that fluorescein-labeled heparin is colocalized with amylin fibrils. The mechanism of binding appears to involve electrostatic complementation between the negatively charged heparin helix structure and the positively charged cross-β sheet structure of amylin fibrils. To see how heparin affects the biological function of amylin, we used a fluorimetric assay of cytotoxicity towards a mouse model of pancreatic β-cells. With heparin fragments longer than 20 saccharides, cell death was similar to when amylin was added alone. By contrast, short heparin fragments of 2 to 8 saccharides protect against cytotoxicity and as such may be leads for drugs to treat type 2 diabetes.

**1228-Plat**

**Investigating the Interaction Between Characterized Amyloid-Beta Oligomers and the Prion Protein Receptor in Live Cells**

**Priyanka Narayan**, Kristina A. Ganzinger, James McColl, Anna Drews, Richard W. Clarke, Seema Qamar, Peter St. George-Hyslop, David Klenerman.
University of Cambridge, Cambridge, United Kingdom.

The devastating symptoms of Alzheimer's disease (AD) have been attributed to the aberrant aggregation of amyloid-β (Aβ) peptides generated from proteolytic cleavage of the transmembrane receptor, amyloid precursor protein (APP). Oligomers of the peptides amyloid-$\beta_{1-40}$ (Aβ40) and amyloid-$\beta_{1-42}$ (Aβ42) have been implicated in cytotoxicity and impaired cognitive function associated with AD[1,2]. Due to their heterogeneous nature, characterizing these oligomers on a molecular level and understanding the mechanism by which they induce cellular damage and death has proven to be difficult for conventional biochemical techniques. In this work, we use two color total internal reflection fluorescence microscopy first to characterize various preparations of Aβ oligomers on a molecular-level and then to explore the interactions of these well-characterized mixtures with live cells. Among the cellular systems examined include those expressing the prion protein receptor (PrPc) which, in recent studies, has been suggested to have a key role in mediating the toxic effects of Aβ oligomers[3]. From these studies we have not only been able to gain insight into the relationship between Aβ oligomers and PrPc on live cells but also have developed a methodology for examination of receptor-mediated Aβ neurotoxicity.

1. Chiti, F. & Dobson, C.M. Protein misfolding, functional amyloid, and human disease. *Annual Review of Biochemistry* **75**, 333-366 (2006).
2. Cleary, J.P. et al. Natural oligomers of the amyloid-β protein specifically disrupt cognitive function. *Nature Neuroscience* **8**, 79-84 (2005).
3. Lauren, J., Gimbel, D.A., Nygaard, H.B., Gilbert, J.W. & Strittmatter, S.M. Cellular prion protein mediates impairment of synaptic plasticity by amyloid-β oligomers. *Nature* **457**, 1128-1132 (2009).

**1229-Plat**

**Gamma Synuclein Forms Tertramers that can be Disrupted by Phospholipase C**

**Urszula P. Golebiewska**, Cassandra Zurawsky, Suzanne Scarlata.
Stony Brook University, Stony Brook, NY, USA.

Gamma synuclein (GS) is a neuronal protein that is expressed at high levels in several cancers and disease states. Like its family member alpha-synuclein, GS is considered to be a natively unfolded protein that can form helical oligomers. Also like alpha-synuclein, the cellular function of GS has yet to be determined. Here, we have characterized the oligomerization of GS using fluorescence homotransfer, photon-counting histogram (PCH) analysis and native gel electrophoresis. We find that purified GS is monomeric on chromatographs under denaturing conditions, but under native conditions it exists as oligomers of varying sizes, with the tetrameric form being the most prevalent. We followed the monomer to tetramer oligomerization by labeling the protein with FITC and following the concentration-dependent loss in fluorescence anisotropy due to homotransfer. PCH analysis, fluorescence correlation measurements and native gel electrophoresis show that placement of the probe and the physical properties of the probe affect the distribution of oligomers. Addition of the enzyme phospholipase Cβ2, a strong GS binding partner whose cellular expression is correlated with GS, results in disruption of GS tetramers and changes in the ability of the enzyme to be activated by G proteins. We propose that one function of GS is to modulate PLCβ2 activity.

## Awards and National Lecture

**1230-Natl**

**Shedding Light on Single Molecules**

**Steven Block**.
Stanford Univ, Stanford, CA, USA.

Technical advances over the past few decades have led to the birth a new field, dubbed ''single molecule biophysics.'' Single-molecule methods can record molecular characteristics that are otherwise obscured using traditional, ensemble-based approaches, and thereby reveal rich new behaviors in biomolecules of interest. An entire arsenal of techniques boasting single-molecule sensitivity has already been developed, including single-channel recording, atomic- and scanned-force microscopy, single-molecule fluorescence and FRET (Förster resonance energy transfer), magnetic tweezers, and more. Prominent among the enabling technologies has been the optical trap, or ''optical tweezers,'' which is based on radiation pressure produced by an infrared laser. When combined with suitable in vitro assays for function, optical trapping microscopes can measure macromolecular properties with unprecedented precision, right down the atomic level (currently achieving a resolution of 1 angstrom over a bandwidth of 100 Hz), all while exerting exquisitely controlled forces in the piconewton (pN) range. Ultrasensitive systems for measuring force and displacement permit the nanomechanical properties of individual biomolecules to be explored noninvasively. Among the more notable successes for optical traps have been measurements of the fundamental steps (and forces) generated by motor proteins and processive nucleic acid enzymes, the strengths of noncovalent bonds between proteins, and the kinetics and energetics of folding in biopolymers, including proteins, DNA, and RNA. This talk will celebrate the success of optical traps in helping to establish single molecule biophysics.