**Purpose of the visit**

The purpose of the visit was to measure the aggregation of amyloid beta in biological fluid. Aggregation of proteins under physiological conditions can lead to disease. Alzheimer's, Parkinson's, Haemodialysis related amyloidosis and many others are related to the formation of protein aggregates. Fibrillation of amyloidogenic proteins is a nucleation process with a lag phase corresponding to the time required to form critical nuclei. Many researchers now feel that oligomers rather than fibrils are responsible for the toxicity associated with these diseases. Fluorescence Correlation spectroscopy (FCS) can be used to study the formation of the early stage oligomers of these proteins. The purpose of this visit was to monitor the diffusion of Amyloid beta monomers and soluble aggregates in biological fluid. N-terminally labelled amyloid beta from commercial sources was used for these experiments. FCS is a particularly powerful technique for these types of measurements, since concentrations of peptide in physiologically relevant regimes can be observed.

**Description of the work carried out during the visit**

Solutions of amyloid beta at a starting concentration of 10μM were mixed with 1% labelled Abeta (HiLyte Fluor) and FCS measurements conducted. The interaction of Abeta with liposomes of various compositions was measured by monitoring the diffusion of the fluorescently labelled Abeta. Initially vesicles composed of POPC with a diameter of 100nm were used, but liposomes containing POPC in mixtures with POPS, sphingomyelin and cholesterol were also investigated. These measurements were deemed necessary before introducing a biological fluid, since Abeta is known to interact with cell membranes. This interaction is important in the context of understanding the aggregation and fibrillation of amyloid beta in a physiologically relevant regime.

**Description of the main results obtained.**

Initially measurements on the diffusion of Abeta alone revealed that there is a significant amount of peptide binding to both glass and polystyrene surfaces,

resulting in heterogeneous Aβ nucleation and subsequent fibrillation. Using polylysine coated chambers prevents this heterogeneous surface nucleation. Using N-terminally fluorescently labelled Abeta 42 (1% labelled in synthetic Aβ, using both HiLyte Fluor and TAMRA labels), we have determined the hydrodynamic radius of the peptide to be ~1nm, indicating that our solution contains mostly monomer. We found significant differences in the binding of Aβ to 100nm extruded vesicles, depending on the lipid composition. There was no binding of Abeta to POPC or 92:8 POPC/POPS vesicles over a number of hours. Binding of Abeta to POPC/POPS/Sphingomyelin and to POPC/POPS/Spingomyelin/Cholesterol vesicles did occur and lead to aggregation/fibrillation of the peptide. Aggregation/Fibrillation occurs over long time-scales (hours to days) and it is unclear as to when peptide binding to the membrane is at equilibrium and how this is affected by the subsequent condensation of the protein. Further work is required to investigate these issues.

**Future Collaboration with Host Institution**

Further discussions with Prof. Joachim Rädler regarding future development of the project took place. These discussions ultimately led to a joint grant pre-proposal being prepared and submitted to support this project in the longer term.