Purpose of the visit

The aim of the visit was to determine the feasibility of using fluorescently labelled monomers of amyloid beta for mechanism studies and to establish an experimental procedure for these and later studies.

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. We aim is to use FCS to eludidate the mechanism of oligomer and fibril formation in the presence of nano-particles. Previous results show that in the case of β-amyloid, polymeric nano-particles inhibit the formation of fibrils, but little is known about the precise mechanism of interaction. FCS is a versatile technique that may help shed light on this mechanism. Using both fluorescently labelled amyloid beta and nanoparticles with different colour dyes on each species will allow us to measure both the individual correlation functions for the particles and a cross-correlation to measure their association over time.

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 and FCS measurements conducted. The concentration of Abeta was varied. Mixtures of labelled (1%) Abeta and 40nm fluorescently labelled nanoparticles were measured using single colour FCS and also in cross correlation mode.

Description of the main results obtained.

It was determined that fluorescently labelled A-beta could be used with unlabelled Abeta at a ratio of 1:100 (labelled:unlabelled) over a range of concentrations (1μM-100μM) for FCS measurements.

The hydrodynamic radius of amyloid beta was measured to be ~ 1nm, indicating that the peptide was mostly monomer. A one component fit was used to determine the diffusion time, and hence the hydrodynamic radius. A two or three component fit to the autocorrelation curve gave no significant improvement.

The dark red polystyrene nanoparticles were found to have a hydrodynamic radius of ~ 70 nm, with a low polydispersity.

The fluorescently labelled amyloid beta was found not to aggregate at concentrations up to 30μM consistent with the work of Rigler et.al. [*Chem & Biol*., **6** (1), 1999]. The fluorescent labels for both Abeta and the nanoparticles were selected to ensure no "cross-talk" would be observed. This was found to be the case.

The starting conditions for experiments on the mechanism for peptide-nanoparticle interactions were established. N-terminal fluorescently labelled Abeta was found to be suitable for fibrillation experiments using FCS.

Future Collaboration with Host Institution

Discussions with Prof. Joachim Rädler regarding future development of the project took place. Having optimized the starting conditions for experiments, future work investigating the mechanism of interaction of peptides with nanoparticles is feasible, and will be conducted through this collaboration.