ESF – Short Visit Grant - REPORT

Visits 17-19 and 23-26 March of Joachim Rädler at UCD

Project: Investigating protein fibrillation in the presence of nanoparticles, using Fluorescence Correlation Spectroscopy.

Purpose of the visits

The short visits are concerned with the investigating protein fibrillation in the presence of nanoparticles, using Fluorescence Correlation Spectroscopy. The project is carried out in collaboration with Kenneth Dawson, Fiona Quinlan-Pluck and Dominic Walsh. The short visits will be used for discussion to establish the framework, experimental conditions and data analysis for this novel approach. Experiments on sample preparation, biochemical modification and particle characterization will be carried out in Dublin, while FCS measurements are carried out in my lab in Munich. The visit is subdivided into two short visits of 3 days each for efficiency in terms of working cycles and cost.

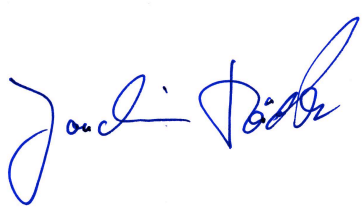
Achievements

The new fluorescence correlation spectroscopy data of Fiona Quinlan-Pluck were discussed. Aggregation of beta-amyloid could be observed in FCS using Thioflavin T (ThT) which binds specifically to amyloid aggregates. No modification of the protein, which could interfere in the fibrillation process is needed. In a first analysis the average size for the growing aggregates can be obtained as the fibrillation process develops. Following the Rigler Paper it might also be possible to determine a size distribution function using an analysis known from light scattering. This approach will be followed up.
Fluorescently labeled polystyrene particles were investigated. Low concentration of particles is needed to not saturate the detector. The particle sizes were found in agreement with manufacture values. In presence of particles, the fibrillation process of beta-amyloid can be followed using two different channels. This possibility allows us to confirm the obtained results regarding diffusion time and therefore size. ThT emits at a wavelength that does not allow cross correlation experiments. A dye emitting at longer wavelength is needed.
Aggregation of particles must be avoided to be able to easily identify interaction between nanoparticles and protein aggregates.

Discussion with Dominic Welsh: Synthetic Abeta is questioned in its role as a clinically relevant model system and the community moves towards looking at extract material from brain (CSF). One way to do so would be to use Fab fragments. Fab

fragments targeting specifically Abeta monomers, as well as dimers, and general Abeta binders exist. The plan would be to label the „general“ Fab fragment with Alexa (488). Fab has 40kD and would be 10x larger than Abeta. With this approach we should be able to detect Abeta aggregation in CSF. This would be first time application of FCS and interesting in itself. Furthermore we can study the interaction with NP.

A second visit in April was arranged to proceed with the protein labeling.



Prof. Dr. Joachim Rädler