ESRF	**Experiment title:** Protein Fibrillation - investigating different species in the assembly process and the effects of hydration	**Experiment number**: MX 1614
Beamline: BM 29	**Date of experiment**: from: 16 March 2014 (09:30) to: 17 March 2014 (08:00)	**Date of report**: 17 June 2014
Shifts: 3	**Local contact(s)**: Martha BRENNICH	*Received at ESRF:*

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Report:

The aim for the data collection in this beamtime allocation was to characterize different specific states of the protein assembly process related to protein fibrillation by SAXS. It is thento be complemented and correlated with other analyses (e.g FTIR, TEM, fiber diffraction, fluorescence spectroscopy, all done within the group in relation to these projects). Several different fibrillating systems are being analyzed.

The allocated shifts were used fully with only minor breaks for cleaning the sample cell. The automated sample changer was used throughout, also for successfully loading more viscous sucrose containing samples.

A modified version of the amyloid-beta peptide is investigated for characterization of the structural species leading to formation of plaques as observed in relation to the Alzheimer's disease. Samples of protofibrils of this modified peptide was characterized, and a putative smaller building block was observed in the SAXS data collected. This new species was observed in a sample not fully seperated from minor fractions of protofibrils/aggregates, thus a confirmation is needed before the full modelling and publication. The sample conditions are therefore being optimized, including tests for possible online-SEC to confirm the characteristics of this species.

The role of cosolvents in protein aggregation was also investigated. Using insulin as a model system, we studied the effect of ethanol on the kinetics of aggregation, including also the final mature aggregate structures. The effect of ethanol on the native state was detected, with a change in the association state of the protein as a function of the cosolvent concentration. Further complementary analysis (CD, FTIR and light scattering) are in progress to confirm the SAXS data. The analysis of the SAXS data during the kinetics revealed a not negligible radiation damage that was not fully removed by adding either DTT or glycerol. However, the information gathered through the SAXS experiment has led to a further optimization of the sample preparation.

We also investigated the effect of osmolytes on the proteins stability. Their effects on conformational equilibria within native-state ensembles of proteins remain controversial, nonetheless is known that they increase the thermodynamical conformational stability of proteins, shifting the equilibrium between native and denatured states to favor the native state. We studied if and how sucrose, a model osmolyte, can influence the fibrillation process and the surface of insulin fibers. A previous analysis of the system by several biophysical techniques (CD, FTIR and fiber diffraction) has shown that adding the sucrose to pre-formed fibers affects their stability but no changes in the secondary structure of the fibers were detected. The preliminary analysis of the SAXS experiment performed show different behavior of the surface of the fibers as a function of the increase of the sucrose amount. These results will be complete with a more accurate analysis and a TEM and AFM experiments.

Finally, we investigated how several mutations in the C-terminal region of alpha-synuclein influence its monomeric state and the amyloid aggregation propensity. Our data reveal that at least two sites in the C-terminal have strong influence on the aggregation propensity. This is already very promising, but in order to fully understand the influence of the mutations, we also need data from the pure species involved in the complex equilibria. We are thus optimizing the experimental conditions and gel filtration protocols to collect the last data for this publication.