ESRF	**Experiment title:** Protein aggregation at lipid membranes – elucidating IAPP fibrillogenesis and its inhibition	**Experiment number**: SC-2709
Beamline: ID10B	**Date of experiment**: from: 01/07/09 to: 07/07/09	**Date of report**: 31/08/09
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Names and affiliations of applicants (* indicates experimentalists):

C. Jeworrek*, K. Weise*, R. Winter

TU Dortmund, Physikalische Chemie I, D-44221 Dortmund

F. Evers*, M. Tolan

TU Dortmund, Experimentelle Physik I, D-44221 Dortmund

Report:

In this study, we investigated the interaction of islet amyloid polypeptide (IAPP) with lipid films spread at the air-water interface by X-ray reflectivity measurements (XRR).Anionic lipid interfaces are known to foster IAPP fibrillogenesis, which is responsible for type-II diabetes mellitus. Moreover, we probed the effect of insulin acting as a natural inhibitor on IAPP fibrillation at lipid membranes. The detailed analysis of the scattering data will yield a detailed molecular-scale picture of the structural transformations occuring during the nucleation, aggregation and fibrillation process of IAPP and its inhibition by insulin at the lipid interface. The results obtained will help understanding the mechanism of the aggregation and inhibition process of IAPP in the presence of lipid interfaces .

In their physiological environment, proteins adopt a functional folded state, which results from highly regulated cellular processes. Upon failure of the quality control of the cell, proteins may suffer from degradation and can form assemblies from unfolded or partially folded monomers or protein fragments, such as ordered cross-ß-sheet rich structures called amyloid fibrils.[1] Misfolding, aggregation, and fibril formation of proteins such as Aß, α-synuclein or IAPP have severe implications in neurodegenerative diseases like Alzheimer's and Parkinson's disease or in affecting peripheral tissues as in the case of diabetes mellitus type II.[1-4] Inhibiting amyloid fibril formation is regarded as a potentially key therapeutic approach toward amyloid-related diseases.[4,5] Although the process of inhibition is not fully understood yet, screening of inhibitors turned out to be beneficial.[4-6] Recently, it has been shown how small-molecule inhibitors [5] and phenolic inhibitors [6] can avoid IAPP fibril formation. Insulin is known to act as a natural inhibitor for IAPP fibrillogenesis.[7,8] Despite recent efforts toward a biophysical characterization of the aggregation and inhibition process, [1-10] the lack of structural information still hampers the understanding of fibrillogenesis and its inhibition.

To reveal structural details of protein-lipid films with Angstroem resolution, resorting to X-ray scattering techniques is very beneficial. In particular, determination of the vertical electron density profile of Langmuir films is made possible by using the liquid surface scattering setup of beamline ID10B for XRR experiments.

In a previous XRR study, [11] we could show that the lipid-induced fibrillation process of IAPP at anionic DOPC/DOPG lipid films is initiated by lipid-induced nucleation, oligomerization, followed by detachment of larger IAPP aggregate structures from the lipid membrane, and terminated by the formation of mature fibrils in the bulk solution. Moreover, the potential of the polyphenolic red wine compound resveratrol to inhibit IAPP aggregation also in the presence of aggregation-fostering negatively charged lipid interfaces was explored.

Hence, we used a biologically more relevant, heterogeneous model membrane system, namely neutral heterogeneous DOPC/DPPC/cholesterol mixtures acting as a ternary model raft system, and studied the interaction of IAPP, insulin and IAPP-insulin mixtures with the raft membrane in order to explore fibrillation and inhibition in the presence of lipid membranes.
The experiments were carried out using the Langmuir trough available at the beamline ID10B. Protein solutions with concentrations of 1 and 10 µM were prepared in 10 mM phosphate buffer solutions. Lipid films were spread on the protein solution in the trough. Then, structural changes were followed by XRR measurements until a steady state was reached which took up to 12 h.

At first, the structure of the lipid raft system was characterized. Data, a preliminary fit and electron density profile are shown in Fig. 1. The model fit is consistent with the experimental data.

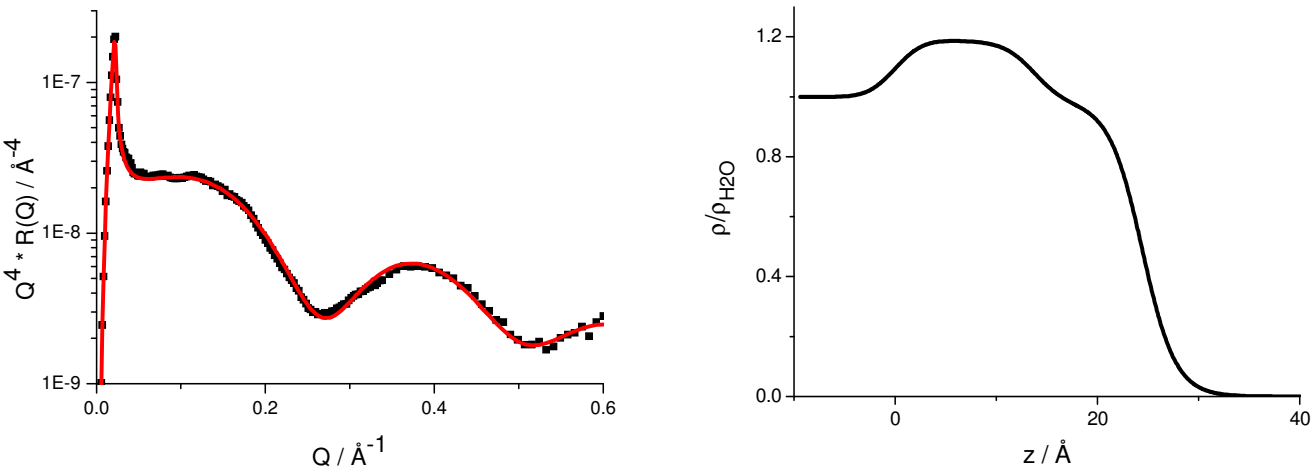

Figure 1. XRR data of the model lipid raft film spread at the air-water interface (left) and the corresponding normalized electron density profile (right).

Figure 2 shows XRR data of the interaction of IAPP and insulin in the presence of a model lipid raft membrane consisting of DOPC, DPPC and cholesterol (IAPP:insulin 1:1 left, 1:10 right). The evolution of the film pressure at a constant compressed area during these measurements is depicted in Fig. 3. We observed clear changes of the reflectivity curve depending on the IAPP:insulin ratio. The detailed analysis of these curves, which is currently performed, will yield valuable information about the IAPP-insulin interactions in the presence of the model raft membrane.

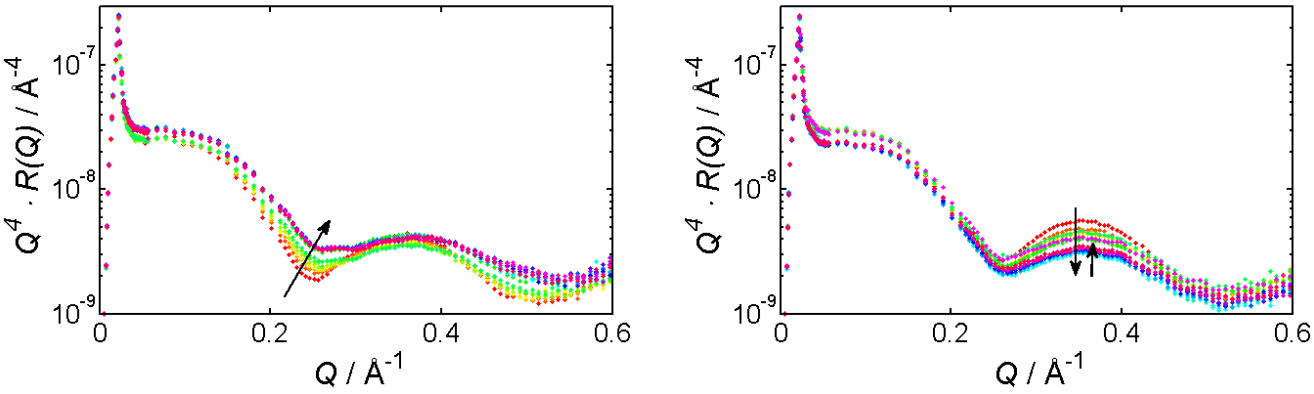

Figure 2. XRR data on the IAPP-insulin cross-interaction in the presence of model raft lipid layers at different IAPP:insulin ratios (1:1 left, 1:10 right). Arrows indicate the temporal evolution.

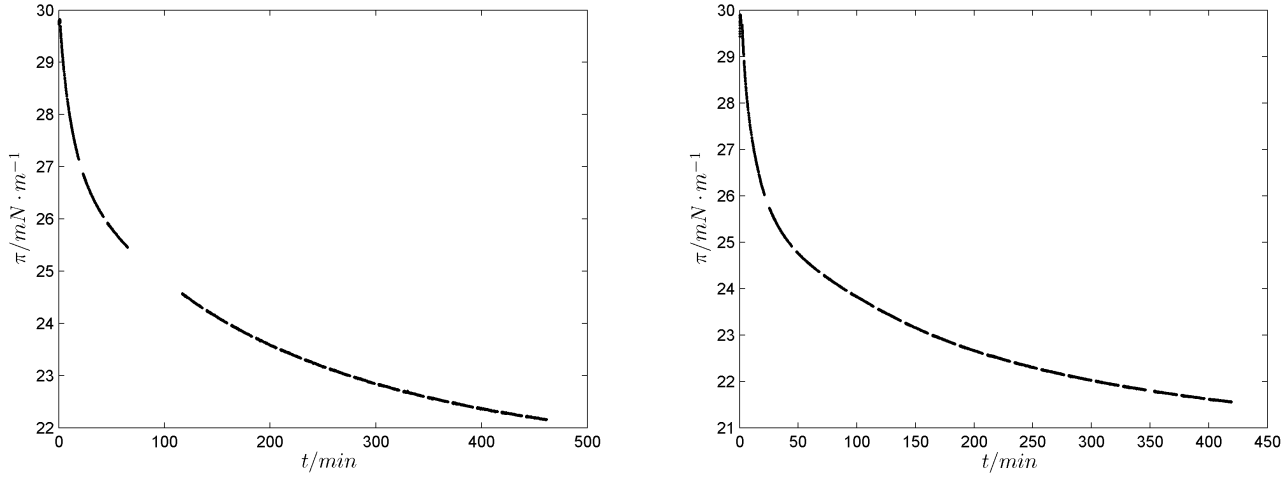

Figure 3. Changes of the film pressure, π, at raft membranes interacting with IAPP and insulin (1:1 left, 1:10 right).

In summary, we successfully measured the interaction of IAPP with model lipid raft membranes and the effect of insulin as a natural inhibitor of IAPP fibrillation by X-ray reflectivity. We obtained systematic XRR data characterizing the interfacial structure of the lipid film and its changes upon interaction with IAPP and insulin. Hence, from the detailed analysis of all data, we will gain a deeper understanding of the mechanisms of IAPP-insulin interactions in the presence of raft membranes as valuable model systems for the complex heterogeneous cellular membranes. A detailed analysis of the data is underway.

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