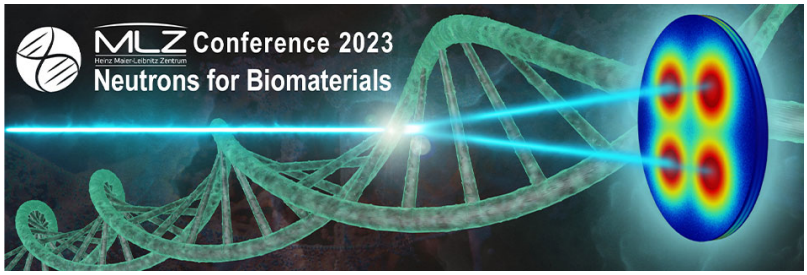

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To pump or not to pump: Combining several scattering and optical absorption methods following the formation of biomaterials.

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In this contribution we discuss the use of a circulating liquid sample pumped by a peristaltic pump through the cuvettes of different scattering or absorption techniques in order to follow processes in biomaterials formation in time. The tested techniques are UV-Visible spectroscopy, Circular Dichroism, infrared spectroscopy or static light scattering, further more we used small angle x-ray or neutron scattering. As a test sample we investigated the formation of amyloid like structures in insulin at pH 2. Using the amide I band, infrared (IR-) spectroscopy can give information on the fold of the protein and also allows to follow aggregation phenomena. Small angle neutron scattering reports on the global structure of proteins in solution and can give information on the shape of growing aggregates or folded proteins in solution. This is why the two techniques deliver complementary information on the observed process. Since the process of amyloid formation is not very reproducible, the results of different techniques cannot be correlated to each other when they are measured one after the other on different samples. Even if one prepares the sample with great care, the lag phase of the amyloid formation is known to be not very predictable.

Furthermore, we would like to explore the capabilities of infrared spectroscopy based on quantum cascade lasers (QCLs) in combination with other techniques. The advantages of QCLs are superior Gaussian beam characteristics and a higher spectral density as compared to the glow bar infrared light sources of the Fourier-transform infrared spectrometer (FTIR). This allows to measure good quality IR spectra within one second. Their disadvantage is the more complicated pulsed mode of operation and the limited spectral width they can cover.

As a first scientific sample, the effect of a pH drop on protein aggregation and amyloid like structure formation in insulin is investigated. Insulin was dissolved in a phosphate buffer, where the pH was adjusted to 2. At room temperature the sample was pumped through varying combinations of flow through cells of the FTIR spectrometer, the QCL, the UV-Visible spectrophotometer and the static light scattering device. Thereby we could follow the amyloid like structure formation on the very same sample using many different techniques in series.

Implications for the requirements on the samples and the processes involved will be discussed in order to widen the application of the used techniques for following the formation of biomaterials in other sample systems.

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