	Experiment title: In situ and in operando investigation of amyloid-beta oligomerization	**Experiment number**: SC3622
Beamline: ID13	**Date of experiment**: from: 24.06.2013 to: 29.06.2013	**Date of report**:
Shifts: 12	**Local contact(s)**: Emmanuela DiCola	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Oliva Saldanha*

Britta Weinhausen*

Viktor Schroeder*

Rabea Sandmann*

Sarah Köster

Report:

The aim of this experiment was to investigate the early steps of amyloid beta (Aβ) aggregation *in situ* using microfluidic devices. Aβ is known to play an important role in Alzheimer's disease and it is currently debated whether the large, fibrillar aggregates are the cause for brain damage or smaller souble oligomers. In particular, not much is kown about the early assembly steps of this peptide. Microfluidic laminar flow mixers provide the possibility to study these first assembly steps, on a time scale of milliseconds to minutes.

We employed specifically designed microfluidic devices (shown in Figure 1a) that are X-ray compatble with Kapton as the window material. Figure 1b shows a close up microscopy image (taken with the beamline microscope) of the device with the flow directions depicted by arrows. The microfluidic devices ensure laminar flow and allow us to access different time points in the aggregation process. X-Ray experiments were performed in transmission geometry with a microfocused beam (5 x 5 μm², EH 2 of ID13). A visible light microscope (calibrated to the focus of the X-ray beam, Figure 1b) was used to position the sample in the beam. During the beamtime, we experienced a very stable and well-aligned setup.

Full length Aβ peptides (1-42) as well as a shorter mutant (4-42) were investigated. A stable laminar flow was established, as can be seen in Figure 1b (green arrow). The peptides were injected into the device in monomeric form. Upon addition of an aggregation buffer from the side inlets and diffusive mixing in the channels, we expect the onset of aggregation. Flow rates were adjusted so as to obtain a stable jet. The

narrower (as determined by the flow rate ratio of main inlet and side inlets; the sheath inlets serve the only purpose of keeping the system from premature aggregation and the channels from clogging) the central peptide stream is, the faster the mixing occurs due to shorter diffusive path lengths . The underlying principle is that the peptide molecules, which are comparably large, diffuse only very slowly out of the central stream while the smaller solutes (salt ions, hydronium, hydroxite) in the buffers diffuse much faster. We can therefore bring the peptide from the initial buffer into a different buffer which leads to a change in pH or ionic strength and thereby initiates aggregation.

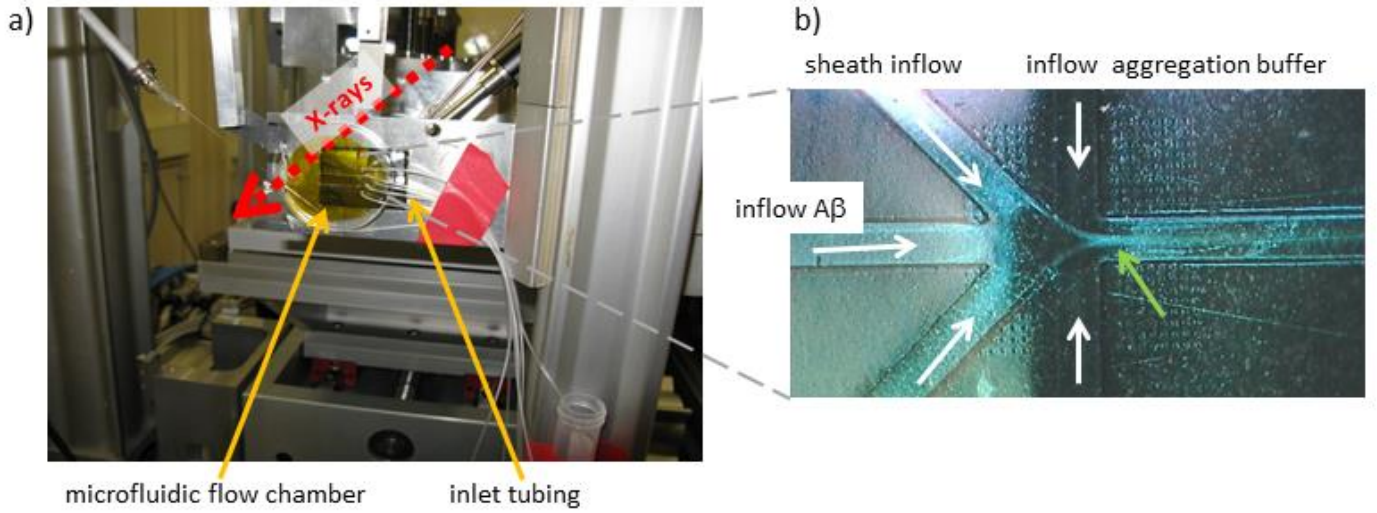

Figure 1. *a) Setup at the ID13 beamline (EH2, microbeam). The microfludic device is mounted on a sample stage for lateral translation through the beam. Inlet tubing is used to inject the diferent fluids into the device. b) Visible light micrograph taken with the beamline microscope. The inflows are depicted by white arrows. The green arrow points to the peptide stream in the center of the device.*

Despite the optically visible stable stream of peptide in the device (green arrow, Figure 1b), the scattering signal from the biological system is hardly detectable. In Figure 2, we show a dark field composite image of the central region of the device. For this dark field image, the device area has been scanned through the X-ray beam in 20-µm-steps. The jet can hardly be detected as the signal is too weak.

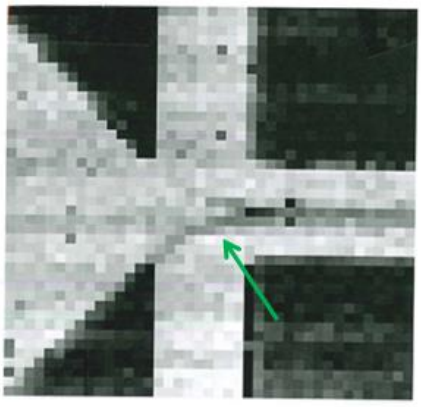

Figure 2. *X-Ray dark field image of the Aβ peptide in the microfluidic device. Even though some of the peptide had adhered to the channel walls, only a very weak signal (green arrow) can be detected. Further downstream (corresponding to later assembly times) we cannot detect a signal at all.*

Our conclusion from this experiment is that the time scales for Aβ aggregation are too long to be detected in our current setup. This is probably due to the fact, that the full length peptide (and even the slightly shorter mutant) needs a comparably long time to rearrange all involved amino acids. Our future experiments will therefore focus on the short FF (di(phenylalanin)) motive which is thought to be responsible for Aβ aggregation and due to its short length assembles much faster and in a more controlled way.