	Experiment title: Kinetics of the early steps in diphenylalanin self-assembly followed by SAXS in microflow	**Experiment number**: SC3834
Beamline: ID13	**Date of experiment**: from: 24.09.2014 to: 28.09.2014	**Date of report**:
Shifts: 12	**Local contact(s)**: Britta Weinhausen	*Received at ESRF:*

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Report:

Motivation: The proposed experiment involved tracking the kinetics of diphenylalanine (FF) dipeptide self-assembly by SAXS in microflow. This idea was based on a collaboration with a group from Tel Aviv University. As this collaboration became less productive, we have redirected our work into studying another biologically relevant system, where our group has gained quite some expertise over the last years, namely vimentin intermediate filaments (IFs). However, the main idea of the experiment remains the same and we could show that combining droplet microfluidics and small-angle X-ray scattering (SAXS) enables us to investigate the initial aggregation stages in biological systems.

Vimentin IFs are mainly found in cells of mysenchymal origin and belong to the cytoskeletal filaments, thus playing an instrumental role in cellular mechanics. As vimentin IFs are highly negatively charged, they posses polymeric and polyelectrolytic properties. Our interest was to access the early stages of vimentin aggregation in the presence of divalent Mg^{2+} ions at millisecond to second time resolution. We employed droplet microfluidics to encapsulate the vimentin networks. Bulk SAXS studies of vimentin filaments and networks in the presence of mono- and divalent ions [1] had been performed previously in standard capillaries and network aggreagtion in micriofluidic drops had been observed by microscopy [2-4].

A PDMS-capillary composite microfluidic device (see figure 1a) was used for the experiments and the devices served two objectives, controlled water-in-oil droplet generation and X-ray compatibility. One great advantage of using microfluidics for studying protein systems and flowing the biological material by the x-ray beam is that radiation damage is substantially reduced.

Experimental setup and data collection: The three aqueous inlets 1,2,3 were used to mix vimentin with the Mg^{2+} ions separated by a stream of buffer. The droplets were then pinched off by the oil phase flow (inlet 4) from adjacent directions [2,4]. These droplets then flowed down the outlet channel into the quartz capillary. Data were taken at several positions along the capillary enabling us to convert the spatial resolution into time resolution and reaching a time resolution of milliseconds. Short exposure times of 10 ms with 3 ms dead time were employed. We used a micro-focused X-ray beam with dimensions of 5 μm x 5 μm which was incident

at different points on the capillary whilst the droplets (dimension of about ~120µm x 80µm (h x v)) flowed by. The data were collected using a PILATUS single-photon counting pixel detector, which can acquire with a high frame rate. Due to the small beam size and the short exposure time, several acquisitions per flowing droplet were possible (~ 22-26 exposures). This strategy allowed us to distinguish clearly between the aqueous (red) and oil (blue) phase (see figure 1b). As the oil absorbs the x-rays more strongly than the aqueous phase, the higher scattering intensity can be attributed to signal from inside the drops. In order to increase the signal-to-noise ratio, we averaged the signal from the aqueous droplets only, while discarding the signal from the oil. The edges of the droplets produce streaks showing us the interface between the oil and droplet (see figure 1c) and the averaged frames between two consecutive streak patterns image the entire droplet end-to-end in the horizontal direction during the course of the flow.

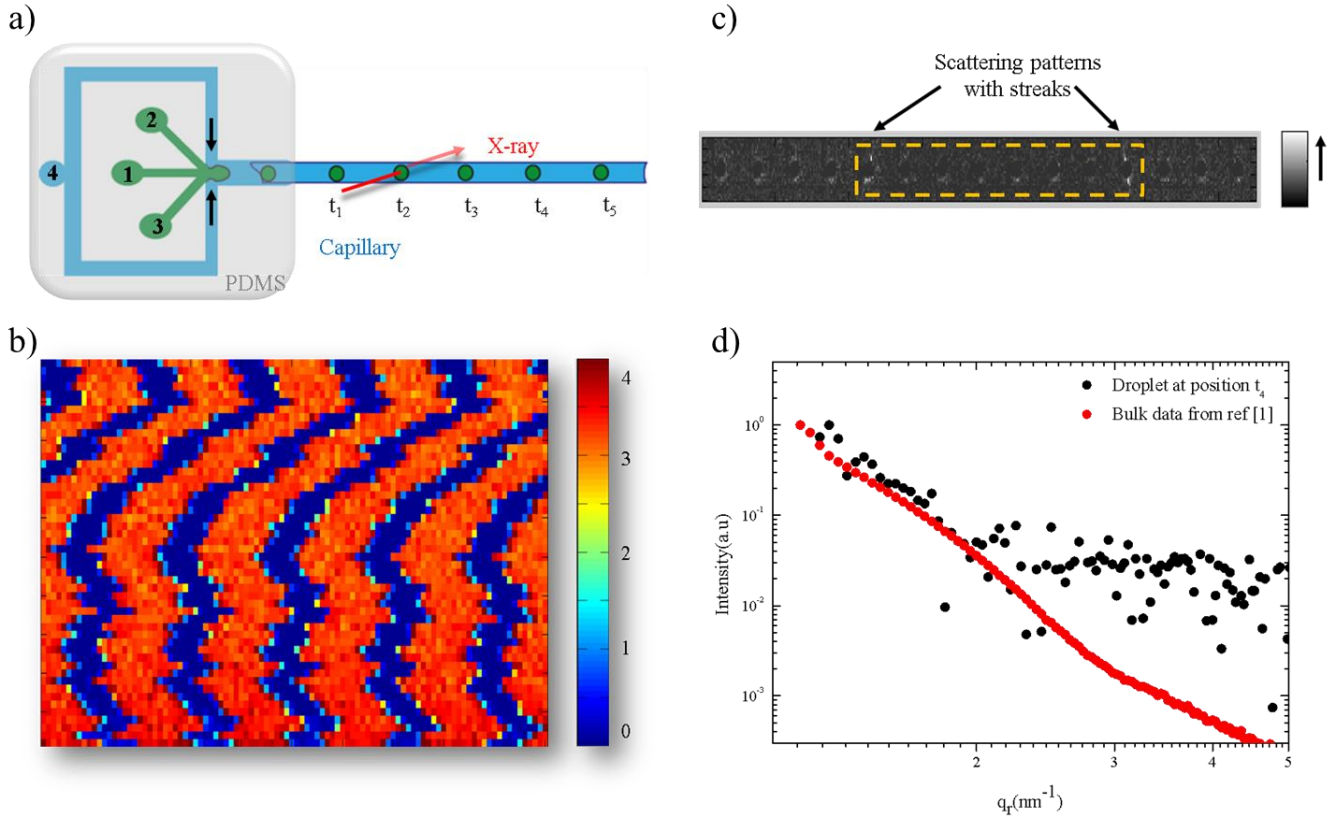

Figure 1: a) Sketch of the microfluidic channel geometry and the PDMS-capillary device for generating water-in-oil emulsions. **b)** Dark field image (individual exposures arranged line-by-line for overview) of the integrated signal from subsequent exposures (10 ms, 3 ms dead time in-between) at one specific point along the capillary. **c)** Composite image of signal taken while one droplet flowed by the beam. The streaks coming from the droplet edges can be clearly seen. **d)** The radially integrated signal (black) was averaged over about 2500 exposures of 10 ms each from inside the drops and background corrected by the averaged signal from empty drops; the data are compared to bulk data (red) [1] at similar salt concentration.

We are currently in the process of analysis of more datasets and the first results look promising concerning our experimental strategy and data collection method. During the beamtime, the support from the staff of ID13 tremendously helped to meet our requirements especially with respect to beam size, *q*-range and computational necessities. From this experiment, we have demonstrated that the study of assembly and aggregation with droplets in flow by x-ray methods is feasible and time scales accessible range from milliseconds to seconds, which is exactly what we need to understand the assembly of biological macromolecules. In addition, the obtained signal is comparable to the bulk data when normalized to the respective integrated intensities and protein concentration (figure 1d). Future experiments will be directed towards implementing the above method for a better understanding of assembly and aggregation of vimentin.

[1] Brennich, M.E., Bauch, S., Vainio, U., Wedig, T., Herrmann, H., Köster, S. (2014) Soft Matter 10 2059 – 2068.
[2] Dammann, C., Köster S. (2014) Lab on a Chip 14, 2681 – 2687.
[3] Dammann, C., Herrmann, H., Köster S. (2014) Israel Journal of Chemistry, (accepted for publication).
[4] Dammann, C., Nöding, B., Köster S. (2012) Biomicrofluidics 6, 022009.