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# Experiment Report Form

**The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF**.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

*http://193.49.43.2:8080/smis/servlet/UserUtils?start*

***Reports supporting requests for additional beam time***

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

***Reports on experiments relating to long term projects***

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

***Published papers***

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

## Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

## Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

| | | |
|---|---|---|
| ESRF | **Experiment title:**<br>Dynamics of Intermediate Filament Self-Assembly | **Experiment number**:<br>SC-2888 |
| **Beamlines**:<br>ID13/ID02 | **Date of experiment**:<br>from: April 9, 2010 to: April, 16, 2010 | **Date of report**:<br>10.8.2010 |
| **Shifts:**<br>12 + 6 | **Local contact(s)**:<br>Manfred BURGHAMMER (ID13),<br>Manuel FERNANDEZ MARTINEZ (ID02) | *Received at ESRF:* |


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## Report:

The aim of this project was the *in situ* investigation of dynamic processes in vimentin intermediate filament self-assembly. Vimentin is a cytoskeletal protein which forms filaments, bundles and networks. These networks contribute greatly to the mechanical and biochemical properties of eukaryotic cells and a detailed knowledge of the assembly mechanism is desirable. In the past, mainly static measurements (using, e.g., electron microscopy or atomic force microscopy) have been performed, revealing snap shots of the assembly intermediates at different stages of the reaction. By performing small angle x-ray scattering (SAXS) measurements of the assembly process within continuous flow microfluidic devices, we overcame this limitation and obtained dynamic data.

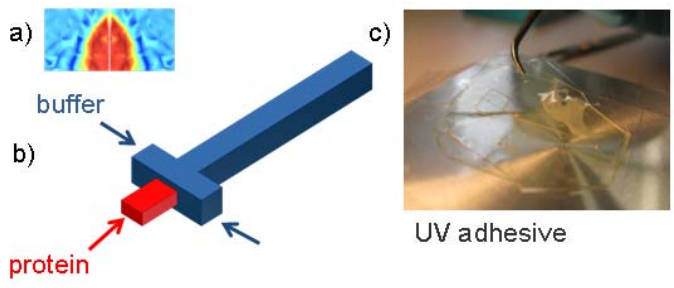


Figure 1: a) Simulation of (upper half) of the main flow channel. High protein concentration is encoded in red, low concentration in blue. b) Schematic 3D view of the flow chamber. The buffer inlet (red) is about half as high as the buffer inlets. c) Photograph of a microfluidic device.

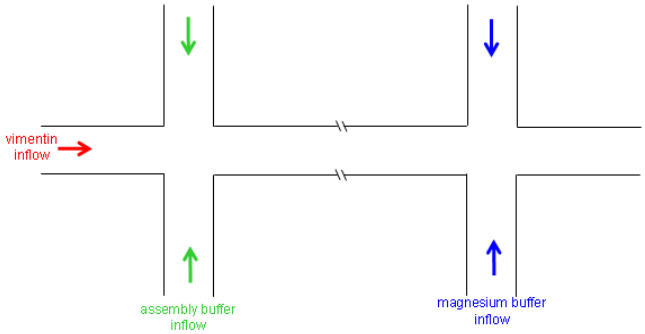


Figure 2: Device design for the experiment with additional magensium buffer inflow further downstream the channel. The first inflowing buffer initiates the vimentin assembly. Once the assembly process is completed (not further changes in SAXS signal), the second buffer containing magnesium is injected.

A special microfluidic flow chamber was developed in which vimentin protein solution (~3 - 4 mg/mL) is hydrodynamically focussed [1,2] by buffer streams from the side containing a high salt concentration. The increase in salt concentration initiates vimentin assembly. The 3D flow chamber (figure 1b) is fabricated in a way that the vimentin stream never touches the channel walls (see simulation of upper half of the channel in figure 1a, high protein concentration is encoded in red, low concentration is encoded in blue) [3]. Furthermore, we used UV curable adhesive, which shows a very low SAXS signal itsself and is at the same time moldable at high resolution (see photograph in figure 1c). We used a channel width of ~300 µm and the resulting protein stream was about 100 µm wide.

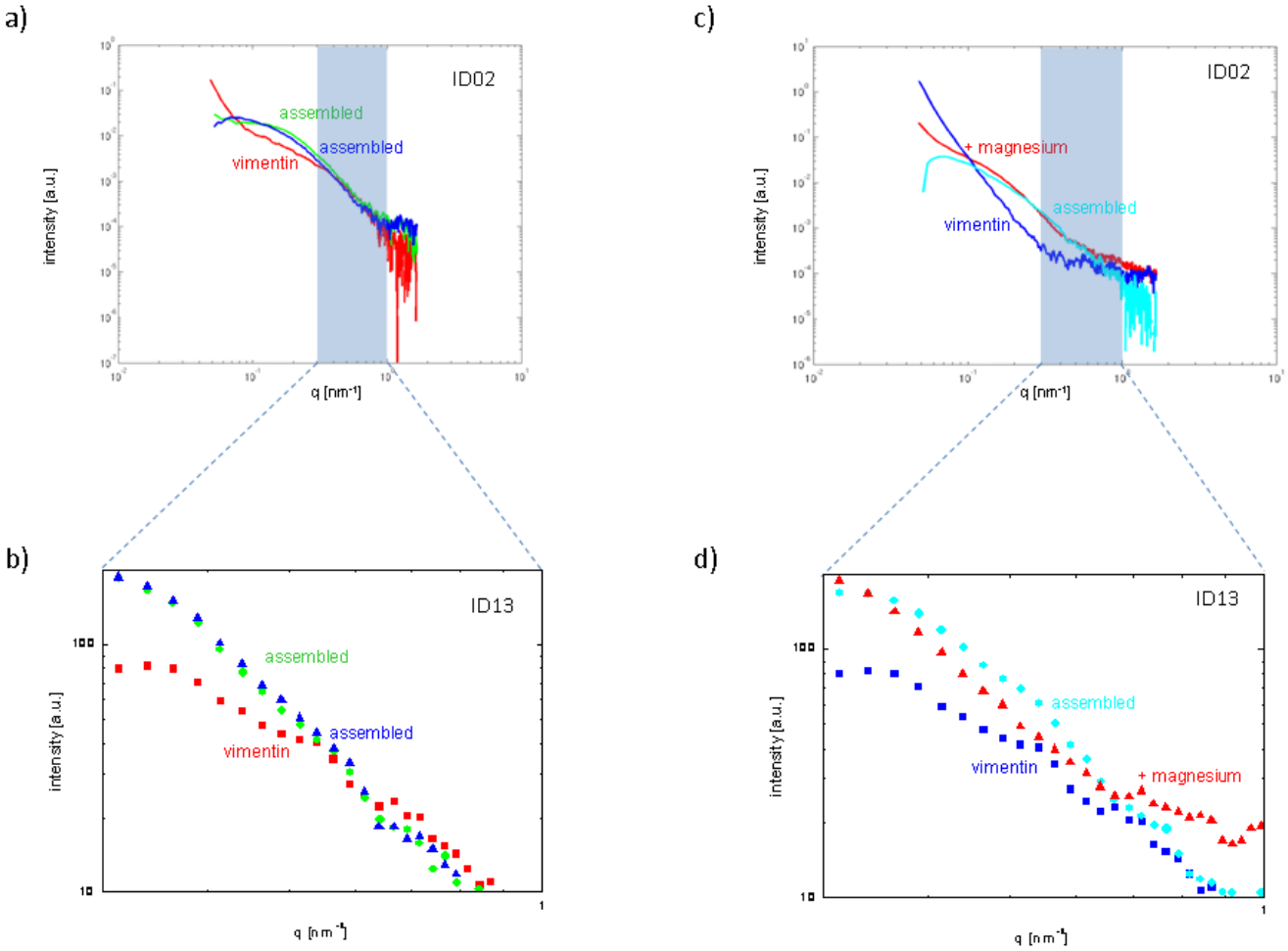


Figure 3: a) ID02 data of the vimentin assembly upon the diffusive mixing with salt buffer. b) ID13 data of the corresponding experiment. c) ID02 data of magnesium addition to vimentin. d) Corresponding ID13 data.

Micro-focused SAXS in combination with microfluidics is a high-resolution technique in two respects. First of all, a microfocused beam with a diameter smaller than the channel dimensions provides a high spatial resolution. The device is „scanned“ through the beam and data from different positions are obtained. Secondly, x-ray scattering itself provides high resolution in q-space.

We used two different beamlines in our experiments, which are complementary concerning the resolution properties. The microbeam at ID13 was focused to a very small spot size (5 x 5 µm²) and thus provided a lateral resolution of 5 µm. Additionally the in-line microscope provided the possibility to exaclty determine where in the flow chamber the measurements were performed. The lateral spatial resolution in a laminar flow mixer as used in our experiments corresponds directly to the time resolution of the experiment. The laminar flow allowed us to use long exposure times (0.8 to 5 s) while fresh material was streaming through the beam. We could thus resolve the reaction kinetic on the second time scale. Since there are no tubulences in the steady state flow, the reaction conditions, local concentrations etc. do not vary over time and data taken from one spot represent an esemble average.

By contrast the beam at ID02 was one order of magnitude larger in each directions (50 x 50 $\mu m^2$) and thus on the same order of magnitude as the protein stream in the flow device. The advantage of this beamline is the extended q-range (0.03 ... 2 $nm^{-1}$) as compard to ID13 (0.3 … 1 $nm^{-1}$). Figure 3a and b show that taken together the data will provide a complete picture of the dynamic assembly process. The ID02 data (figure 3a) cover a large q-range and show emerging features in the SAXS signal during the assembly process. Clearly the assembly kinetics can be followed *in situ* using this method. A complete analysis and interpretation of our ID02 data is currently not yet possible for a technical reason: During our measurements at ID02 accidentally a q-dependent factor had been applied to our raw data. This mistake was only discovered after the beam time and the recovery of the original data by the beam line staff is still in progress. The ID13 data of a very similar experiment (flow rates and exact channel positions vary slightly) are shown in figure 3b. In the overlapping q-range the data are consistent and ID13 offers the additional possibility to analyze data points from more (and more closely spaced) positions in the channel (data not shown).
In addition to the assembly process of vimentin, which was initiated by the inflow of salt buffer, we were able to test the additional inflow of divalent ions, in this case magnesium ions ($Mg^{2+}$). This inflow was located at a position further downstream in the channel. A device similar to the one shown in figure 1b was used, but with additional side inlets (see sketch in figure 2). This experiment goes beyond what was proposed for this beamtime and was a first test of the effect of multivalent ions on the SAXS signal of intermediate filament protein. The promising results convinced us to continue these studies in the future. Again, the overlapping q-range shows agreement between the measurements at ID02 and ID13 further validating the method and the setup. Further data analysis and interpretation is necessary and will be performed as soon as we can work with the original data for our measurements at ID02.

References:

[1] S. Köster et al., Biomacromolecules 9, 2008, 199
[2] S. Köster et al., Langmuir 23, 2007, 357
[3] M. Brennich et al., *in preparation*