ESRF	**Experiment title:** Assessing early steps in keratin filament assembly by microfluidics and SAXS	**Experiment number**: SC4406
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Shifts: 15	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Chiara Cassini*

Manuela Denz*

Sarah Köster*

Eleonora Perego*

Andrew Wittmeier*

Report:

Overview: In this experiment we aimed at combining microfluidics with small angle x-ray scattering to follow the reaction kinetics in protein assembly. Microfluidics provides time-resolved data of such processes. A further advantage of the continuous flow is that radiation damage is greatly reduced as the material is constantly replenished. One key point in the experiment was the design of suitable microfluidic devices and the choice of window materials that ensure low background, low absorption and that are compatible with the biological material. The experiments were performed at ID13, where we characterized two different device types, (i) a design consisting of two 8-µm Kapton films sandwiching the channel-defining layer of a UV-curable glue (NOA81) and (ii) a design consisting fully of Topas (a cyclic olefin co-polymer, COC). For this characterization we used gold colloids with a diameter of 20 nm as a bench mark system. Furthermore we investigated the assembly of the vimentin intermediate filament (IF) protein in the Kapton-window-device.

Experimental setup and data collection: The experiments were performed at ID13/EHII using a micro-focused beam (2.7 x 1.7 μm^2 horizontal x vertical). During the colloid measurements the colloids were entering the device through the central inlet, whereas the other four inlets contained PBS buffer (see sketch o the device in Figure 1A). During the protein measurements, the protein was injected into the central inlet and was mixed with KCl injected from the side inlets. KCl induces the assembly of the IFs. By scanning the central channel we could probe the mixing region and different positions further downstream. An Eiger 4M detector was placed at a distance of 0.95 m in the transmission geometry for SAXS signal detection. Sample alignment was performed using a visible light microscope.

Results

Figure 1 shows the results we gained by comparing the two device types. Analysis of the protein data is still in progess, but not yet included here. In Figure 1B the setup at ID13 including our microfluidicsystem is shown. The data in Figure 1C and D are plotted on a double-logarithmic scale (radial Intensity *I* versus the scattering vector *q*) and show the scattering of gold colloids (20 nm diameter). In Figure 1C radially integrated and background (buffer measurement performed before the actual data acquisition) subtracted data from four different positions along the outlet channel are plotted (see colored dots in Figure 1A), showing the well-known signal for spherical colloids. Figure 1D shows a comparison of the radiallly integrated and background subtracted data from both device types and the calculated form factor for solid spheres. Most importantly, there is no detectable difference in quality between the data set for the different devices. Kapton is widely used as a window material in x-ray experiments, whereas Topas is not yet as established. The device design and fabrication of the all-Topas devices is promising as there is no gluing of different materials involved and no interfaces, thus preventing leaking and failing to a great extent.

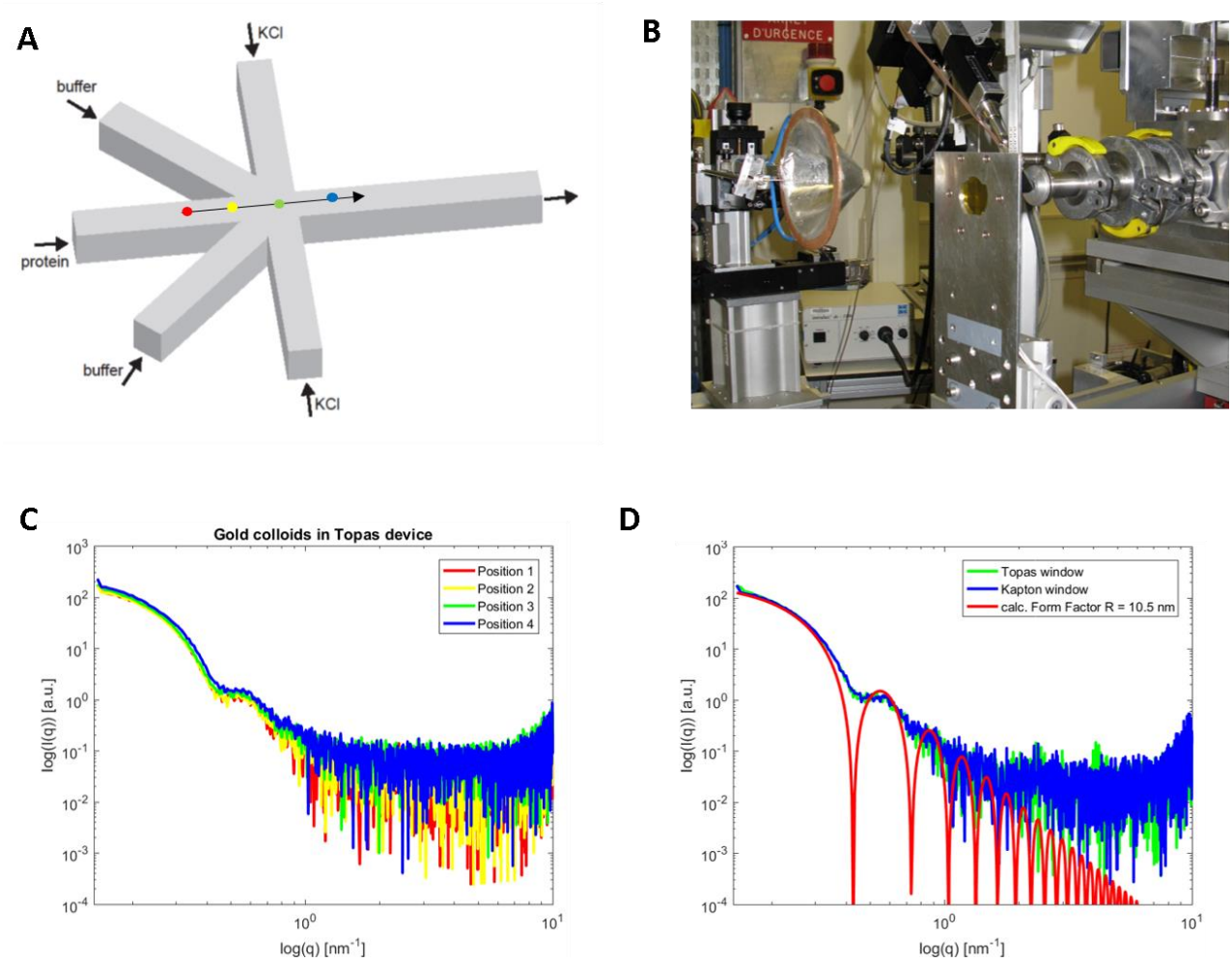

Figure 1: A) Scheme of the device geometry, including five inlets and one outlet. **B)** Photograph of the set-up of a device and sample holder at ID13. **C)** Scattering plots of gold colloids with a diameter of 20 nm in a microfluidic device made of Topas COC. In total 31 positions where analyzed, however here only four are shown. **D)** Intensity plots of gold colloids in a Topas device (green) and a NOA81-Kapton device (blue). The calculated form factor for solid spheres is shown in red.