	Experiment title: **Electric field induced structural perturbations of microtubules probed using difference SAXS**	**Experiment number**: LS-3083
Beamline: ID02	**Date of experiment**: from: 29-04-2022 to: 02-05-2022	**Date of report**:
Shifts: 9	**Local contact(s)**: Lauren Matthews	*Received at ESRF:*

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Report:

Our primary objective with the experiment was to investigate possible structural perturbations of microtubules by high strength electric fields (EF). For this end we had commissioned a purpose-built high electric field generator (**Figure 1**) capable of delivering oscillating electric fields up to 800 V peak-to-peak, and designed a 3D-printed flowcell for the application of an electric field onto microtubules flowing through a capillary (**Figure 2**). We collected datasets for a range of electric field frequencies in the 100 kHz domain. We also investigated if there was any alignment effect on stationary microtubules. However, we failed to detect any structural perturbations nor any alignment effect. This null-result is however interesting, because there have been decades of speculations regarding the special electromagnetic properties of microtubules.

Figure 1: High voltage generator.

Figure 2: Flow cell for the electric field delivery device mounted in hutch.

Our backup objective was to investigate a pH-sensitive potassium channel, KcsA. For this project we had synthesized a caged-compound that breaks down and releases protons, thereby decreasing the pH of the buffer upon illumination. We also designed a 3D-printed flowcell for the illumination of a mounted system using 4 LED diods connected to optical fibres (**Figure 3**).

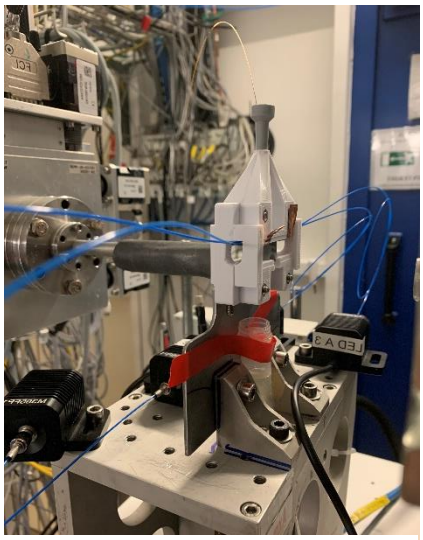

Figure 3. Flow cell for illumination of Serial-X capillary

The photo-release of caged protons worked and we could produce a clear difference signal for the protein altering it's confirmational state (**Figures 4 & 5**). Unfortunately, we found that the pH jump generated a lot of precipitations in the capillary, growing from the X-ray beam spot, which is also indicated as aggregation in the low q-domain in the I vs q plot.

Since this was the first time this protein and the cage compound was used at a beamline, we had to test a range of protein concentrations between 8 and 16 mg/ml to identify the lowest protein concentration with an acceptable signal to noise ratio. We also investigated the different precipitations we got from the protein and the caged compound separately

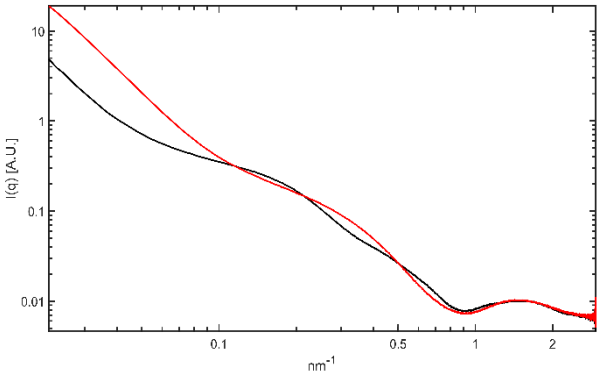

Figure 4: KcsA: black: dark-state, red: pH-shifted state

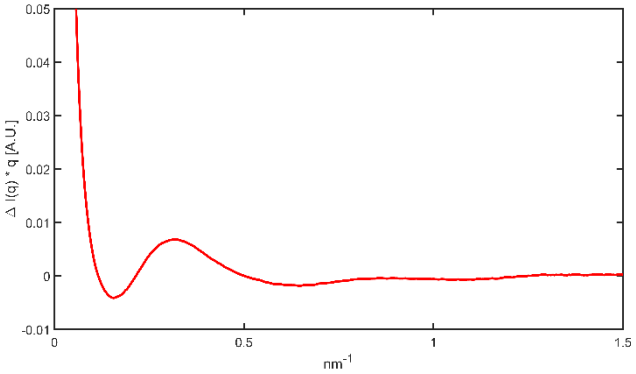

Figure 5: KcsA: Difference curve, light-dark

as well (**Figure 6**). In order to reduce precipitation, we tested a range of concentrations for the caged-compound and the light flux of the LEDs. This information will guide our inhouse optimization of the pH-jumpo system for further experimental studies. We need to find the right ratio of illumination and concentration of the caged compound for us to control the pH, while keeping the concentrations of all species as low as possible to minimize precipitation. We also performed a sequences of increasing flowrate. Since the UV light illumination spot was situated before the X-ray measurement spot, we could in this way achieve a time series of the conformational shift. **Figure 7** shows parts of such a time-series, where the lowest flowrate gives us the full conformational shift (red), while the fastest flow-rate (dotted black) shows the same state as the initial state (black) since the protein has not had the time to undergo a shift. The middle flow-rate (green) shows us a protein in the middle of a shift. Unfortunately the precipitation problem hindered us from using these curves for structural fitting. The time-resolved structural studies needs to be redone after the precipitation problem is solved.

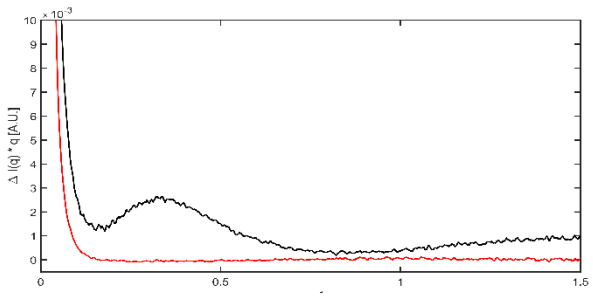

Figure 6: Difference curves indicating precipitation, light-dark (black: KcsA, red: caged compound)

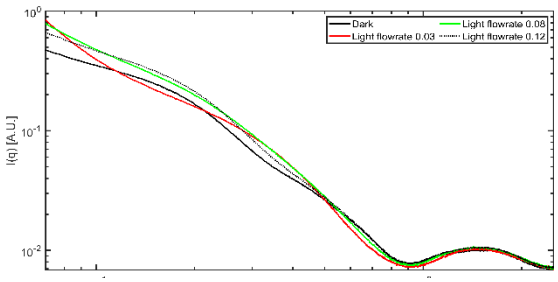

Figure 7: KcsA flowseries