	Experiment title: Ion-mediated organization and structure of keratin bundles studied in vitro by x-ray nano-diffraction	**Experiment number**: SC3960
Beamline: ID13	**Date of experiment**: from: 15/11/2014 to: 18/11/2014	**Date of report**:
Shifts: 9	**Local contact(s)**: Manfred Burghammer, Britta Weinhausen	*Received at ESRF:*

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Report:

Motivation: The proposed experiment involved studying the ion induced bundling in keratin intermediate filaments. Keratin proteins belong to the intermediate filament (IFs) protein family that constitutes the cytoskeleton of eukaryotes together with actin filaments and microtubules. In cells, keratin bundles (laterally associated, packed filaments) provide a prime biophysical example of a highly ordered natural structure. Epithelial cells are continuously exposed to pressures and shear forces and these impacts are to a great part being born by the keratin network within the cells. We formed the bundles by adding multivalent ions to the keratin [1] and used a nano- or micro-focused beam (ID13) to locally probe the structure, orientation and characteristic length scales of the material. We have previously measured keratin in whole, freeze-dried, chemically fixed, or living cells [2-4] and could relate the signal to these keratin structures; however, investigating a corresponding purified system will help to better understand and "calibrate" the signal we have measured in cells.

Experimental setup and data collection: *In vitro* keratin bundles/networks complemented with salt were investigated. The protein was assembled on silicon nitride (Si_3N_4) or TOPAS (a cyclic olefin copolymer) films by droplet-fusion technique. A droplet of protein solution was deposed on one film, a second drop of assembly buffer (containing KCl and/or $MgCl_2$ at different concentrations) was deposed on a second film. The two droplets were then merged by bringing the films into contact [1]. Bundles were localized using the online microscope of the beamline (Fig. 1 a and e), and we then scanned the sample through the beam. We had performed test experiments during a previous beamtime (SC3834, at ID13 EH2) with a micro-beam (5x3 μm^2 horizontal x vertical) and a step size of 1 μm and now repeated this experiment with a smaller beam and smaller steps sizes.

Results: Using the microbeam, we could record dark-filed images of the bundles (Fig. 1 b and f) and detect anisotropic scattering signal from single diffraction patterns (Fig. 1 c and g). We analyzed the data to find the orientation of the local structures and the degree of anisotropy (Fig. 1 d and h). Subsequently, we decided to perform experiments at the nano-hutch with a smaller beam (100 x 150 nm^2) and smaller steps size (100 nm) in order to increase the spatial resolution in real space.

Surprisingly and contrary to the results with the micro-beam, we were not able to collect any signal from the keratin bundles with the nano-beam. One explanation is that at the nano-hutch the beam was 100 x150 nm^2 for a primary beam intensity of about $3x10^9$ photons s^{-1}, thus a flux of about $2x10^{23}$ photons $s^{-1}m^{-2}$. For the beam used in the micro-hutch the flux was about $6x10^{21}$ photons s^{-1} m^{-2}. Thus, the energy density is much higher in the case of the nano-beam than the micro-beam. Therefore, we hypothesize that we destroyed the protein structures on the samples before collecting any diffraction signal. Moreover, the energy at the nano-hutch (14.9 keV) was slightly greater than the one in the micro-hutch (12.5 keV).

Although no conclusion can be drawn from the nano-hutch experiments, from the experiments performed at the micro-hutch, we were able to collect were data for several good samples at the micro-hutch. With the data analysis lined out above, we are confident to be able to show structural changes induced by different ions and different concentrations (see Fig. 1 a-d for one example of keratin bundles assembled in the presence of 20 mM KCl and Fig. 1 e-h for keratin assembled in the presence of 1 mM $MgCl_2$). One can see that in presence of 20 mM KCl the anisotropy of the signal is present for each scan point (Fig. 1 c) which is not the case for the sample assembled in the presence of 1 mM $MgCl_2$ (Fig. 1 g), especially in the denser parts of the dark-field image (high intensity, shown in red in Fig. 1 f). Moreover, filaments looks thicker for this concentration of magnesium as compared as the sample with potassium. This aspect could explain the loss of anisotropy which is in fact averaged over several local orientations. Further analysis such as the 1D azimuthally integrated data will reveal the internal structure (e.g. form and structure factors such as cylinders packed into a hexagonal lattice) of the bundles. Further experiments at the micro-branch of ID 13 will be necessary for a comprehensive study of the structures evolving in keratin in the presence of different species and concentrations of ions.

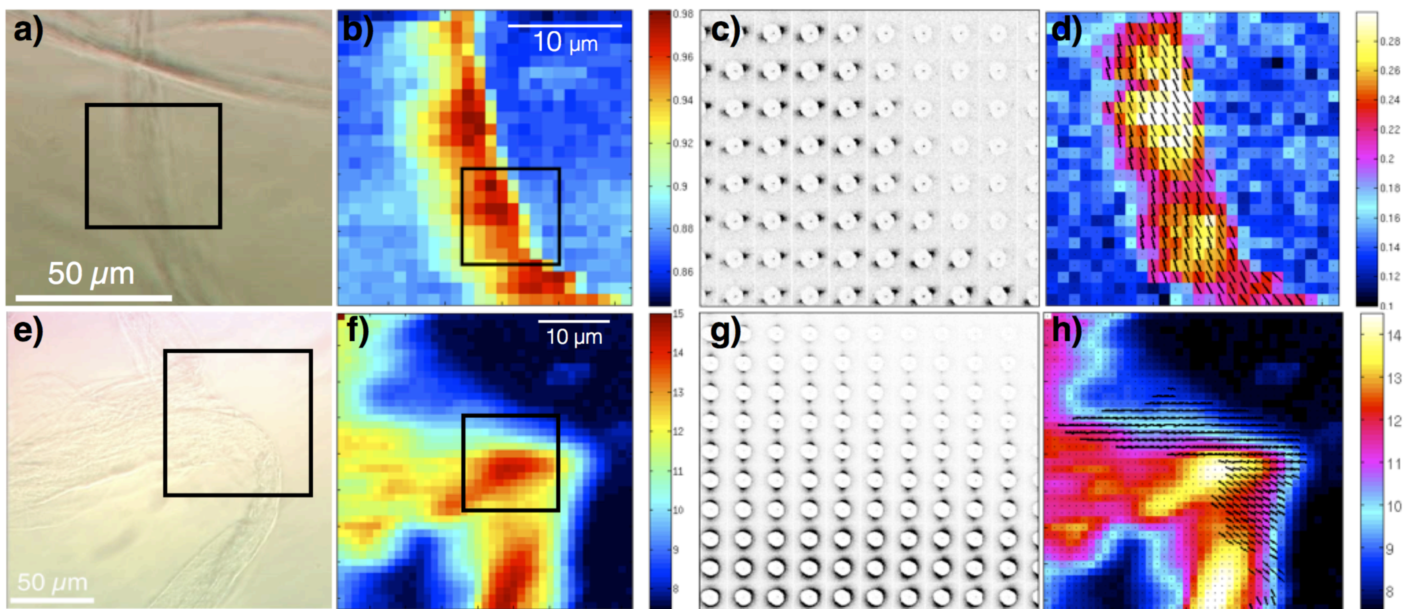

Figure 1: Micro-beam results a) Online micrograph of keratin bundles assembled with 20 mM KCl. **b)** Dark-field representation, of the same bundle. **c)** Composite images of the previous black square from b) showing anisotropy of the signal. **d)** Orientation (vectors) and degree of anisotropy (colormap). **e)** Online micrograph of keratin bundles assembled with 1 mM $MgCl_2$. **f)** Dark-field representation, of the same bundle. **g)** Composite images of the previous black square from f) showing some anisotropy of the signal. **h)** Orientation (vectors) and degree of anisotropy (colormap).

References

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