



	Experiment title: Anomalous x-ray scattering at the sulfur edge of hard alpha-keratin fibers. Technical assessment of diffraction measurements on ID21.	Experiment number: SC-775
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Report:

The aim of the experiment was to test the feasibility of diffraction and microdiffraction experiments at the sulphur K-edge at ID21 beamline, working on hair samples which are known to contain two phases, one characterised by a sulphur content similar to those observed for most proteins and the other being characterised by a very high sulphur content. ID21 beamline is a X-ray microscopy beamline dedicated to medium energy X-ray micro-fluorescence and micro-XANES imaging with submicrometric beams. It is therefore not designed for diffraction data collection and our experiment was the first one proposed in that domain at ID21 beamline.

The scientific background of the experiment was the following. Hair cortex contains two types of proteins. The first type is keratin which are 450 Å long molecules characterized by an alternation of coiled coil parts and non-helical segments. Keratin molecules are assembled both longitudinally and laterally, forming a very complex cylindrical object about 75 Å in diameter, called intermediate filaments (IFs). The IFs are packed into a hexagonal two-dimensional quasi-crystalline array and other proteins, called Keratin-Associated-Proteins (KAPs), are filling the space between the IFs. The KAPs are forming a continuous medium and the IFs are embedded into this medium (matrix). The sulphur content of the matrix is very high, it contains about 25% sulphur atoms, whilst the IF sulphur content is close to 5%. The idea was to benefit from this natural sulphur content contrast to try to get information about the molecular organisation of the KAPs into the matrix, organisation which remains mysterious and subject to controversy despite the important role of the whole tissue hardening due to matrix disulphide bonds. The objective of the experiment was to collect 2-D scattering patterns just above and below the absorption sulphur K-edge to reveal the respective scattering contributions from the IFs and from the KAPs.

The first part of the work consisted in adapting the ID21 setup to diffraction. This was achieved by installing an image plate detector perpendicular or tilted with respect to the incident beam in order to explore various

scattering angles ranges (readable on a STORM scanner from MOLECULAR DYNAMICS). In addition a small beamstop was added to the setup which diameter was small enough to collect SAXS data up to 0.0065 \AA^{-1} , *i.e.* to record the scattering peak arising from the hexagonal IF array. The whole scattering setup was installed inside the vacuum chamber of ID21.

The second part of the experiment was focused on the scattering feasibility on hair. It appeared that diffraction with a 2.5 KeV sub-micrometric beam lead to important radiation damages after a few tens of seconds, preventing to collect diffraction patterns in micro-beam mode. It was then decided to take out the Bragg-Fresnel zone-plate and to work with a less intense beam, ca. 150 \mu m large, intersecting three hairs. Ten to twenty minutes long exposures gave enough intense patterns and the radiation damage was negligible.

Figure 1 shows the SAXS equatorial profiles collected on the two sides of the sulphur K-edge. The intensity peak at about 0.01 \AA^{-1} , which corresponds to the first order reflexion of the IF hexagonal lattice is strongly decreased just above the K-edge whilst the peak at 0.022 \AA^{-1} , which is due to lipids, is unaffected. This result proves the sensitivity of the method for hair analysis, the sulphur contrast strongly decreases just above the K-edge, resulting in the vanishing of the 0.01 \AA^{-1} equatorial peak. Unfortunately the other reflexions at higher angles, in particular the equatorial one at 0.1 \AA^{-1} and the meridional one at 0.19 \AA^{-1} , could not be clearly observed above the K-edge because of their low intensity compared to the huge fluorescence background.

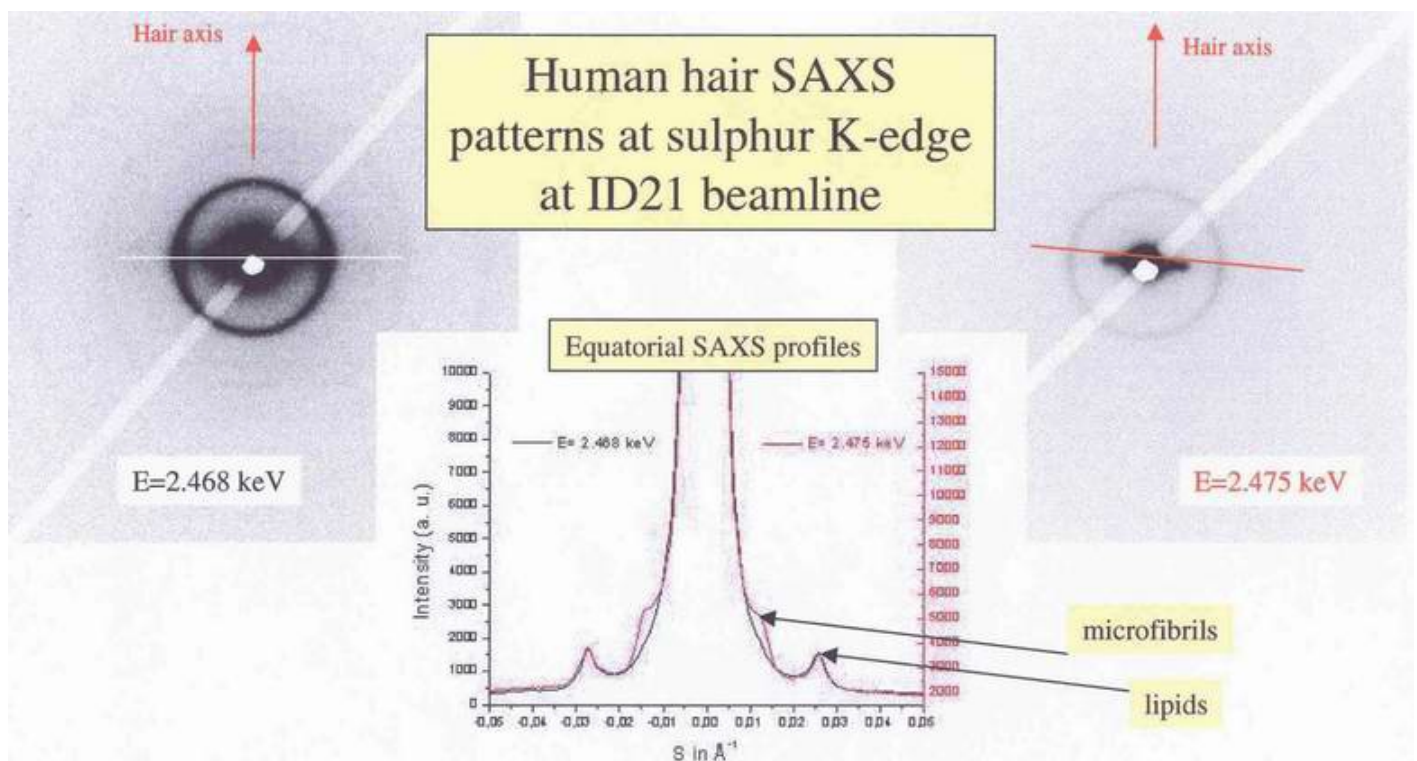


Figure 1: two diffraction patterns collected on the same sample, below (left) and above (right) sulphur K-edge. The comparison of the two equatorial profiles (bottom) exhibits an important variation of contrast in the microfibril SAXS region.

It was concluded that the experiment was feasible but that it was not possible to collect WAXS data above the K-edge. It was therefore envisaged to continue this experiment performing anomalous data collections below the K-edge.