	Experiment title: Alpha-synuclein aggregation mechanisms and conformational changes in presence of ultra-wetting and superhydrophobic surfaces	**Experiment number**: SC4088
Beamline: ID21	**Date of experiment**: from: 17/06/2015 to: 21/06/2015	**Date of report**: 08/09/2015
Shifts: 12	**Local contact(s)**: Dr. Marine Cotte, Dr. Bernhard Hesse (ID21)	*Received at ESRF:*

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Report:

We tested for the first time the X-ray microdiffraction endstation of the ID21 beamline in order to investigate the structure of Alpha-synuclein protein. This peptide is a presynaptic protein (140 amino acids, 19-20 kDa apparent molecular weight) involved in the pathogenesis of several neurodegenerative diseases. It is generally considered an intrinsically unstructured protein and plays a crucial role in the formation of Lewy bodies and Lewy neurites, the cytopathological hallmarks of Parkinson's disease (PD), and related disorders called synucleinopathies. In the specific case we focused our attention on the E46K mutation considering that Familial parkinsonism and dementia with cortical and subcortical Lewy bodies were found to be associated with a 14,460 Da mutated form of alpha-synuclein. This mutation is a 188G-A transition resulting in a Glu46-to-Lys (E46K) substitution in the amino-terminal region of the protein. Among the familial mutations of a-synuclein in PD, E46K has the greatest potential to aggregate. We dissolved the above mentioned peptide in deionized water at a final concentration of 1 mg/ml. Following a well established protocol we then dried µL droplets on a superhydrophobic substrate in order to obtain a freestanding residue (Figure 1A). The residue

was then mounted on a glass capillary and consequently on a motorized stage in the XRD experimental hutch of ID21 (Figure 1B).

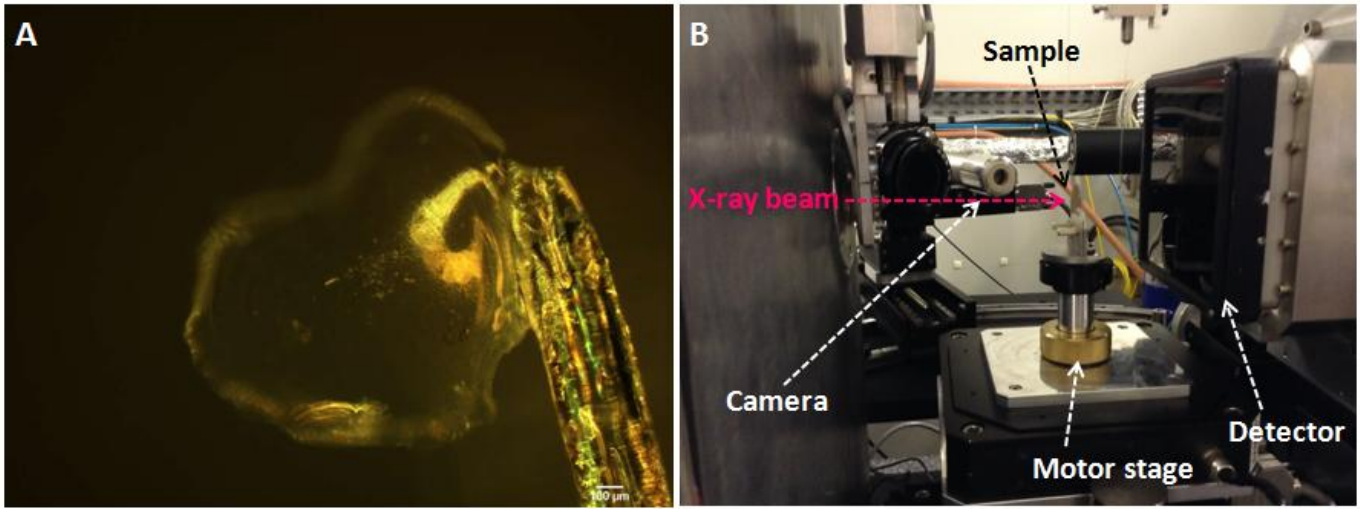

Figure 1. A: Free-standing residue of E46K Alpha-synuclein peptide dried on a superhydrophobic substrate and mounted on a glass capillary tip; B: experimental setup of the brand-new micro-XRD endstation of ID21.

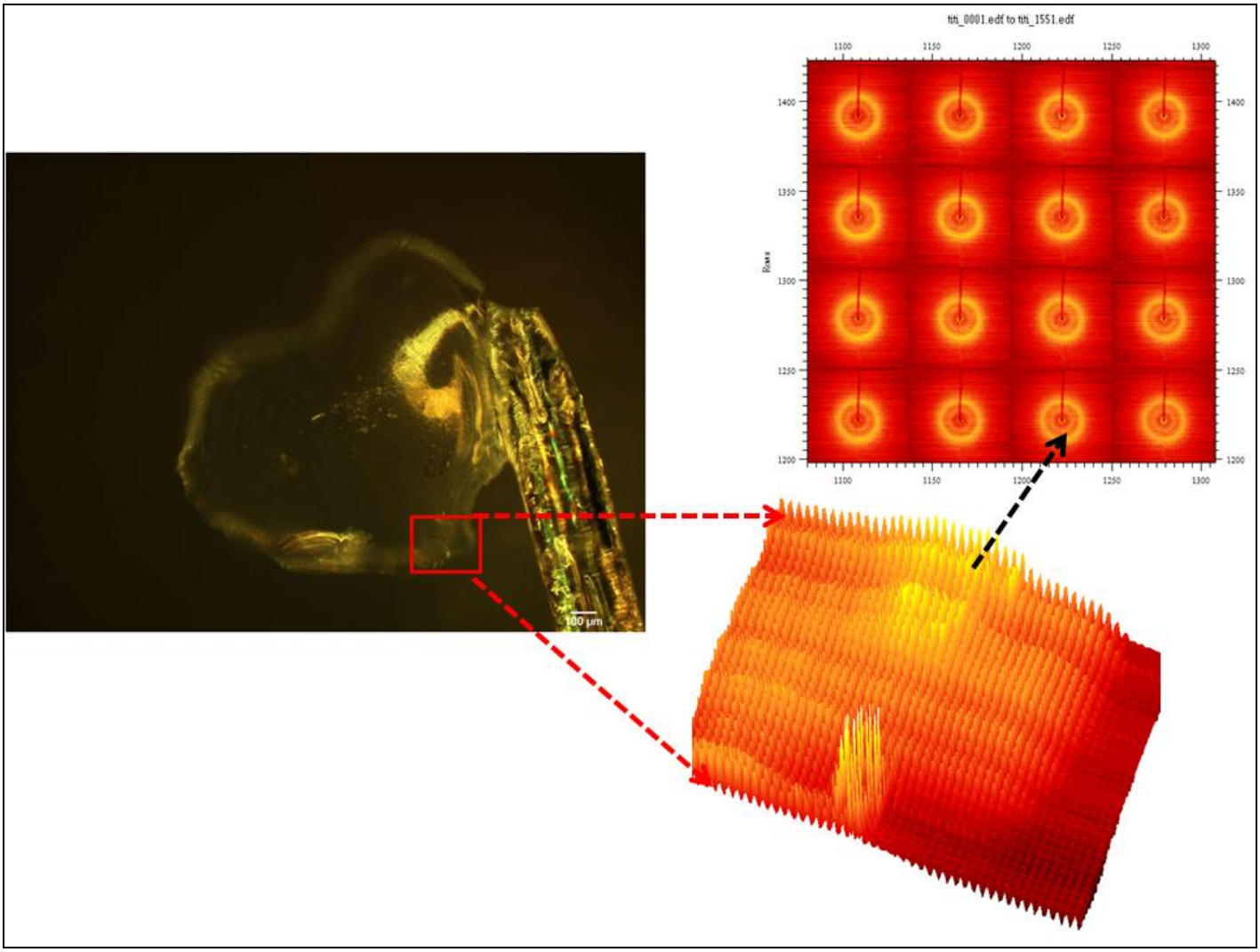

Figure 2. X-ray diffraction mesh scan of a region of the E46K residue obtained by Fit2D and typical XRD patterns.

The X-ray setup was aligned by the beamline staff to the following parameters according to our needs: beamsize 0.6 (hor) X 1.5 (ver) μm^2, E=8.525 KeV, λ=0.145 nm. Figure 2 represents an XRD mesh scan,

obtained by Fit2D, of 47 (hor) X 32 (ver) points of the area covered by the redsquare in the optical image. The possibility of making such composite XRD image was allowed by the user graphical interface of the beamline and by the presence of a lateral camera (Figure 1B).

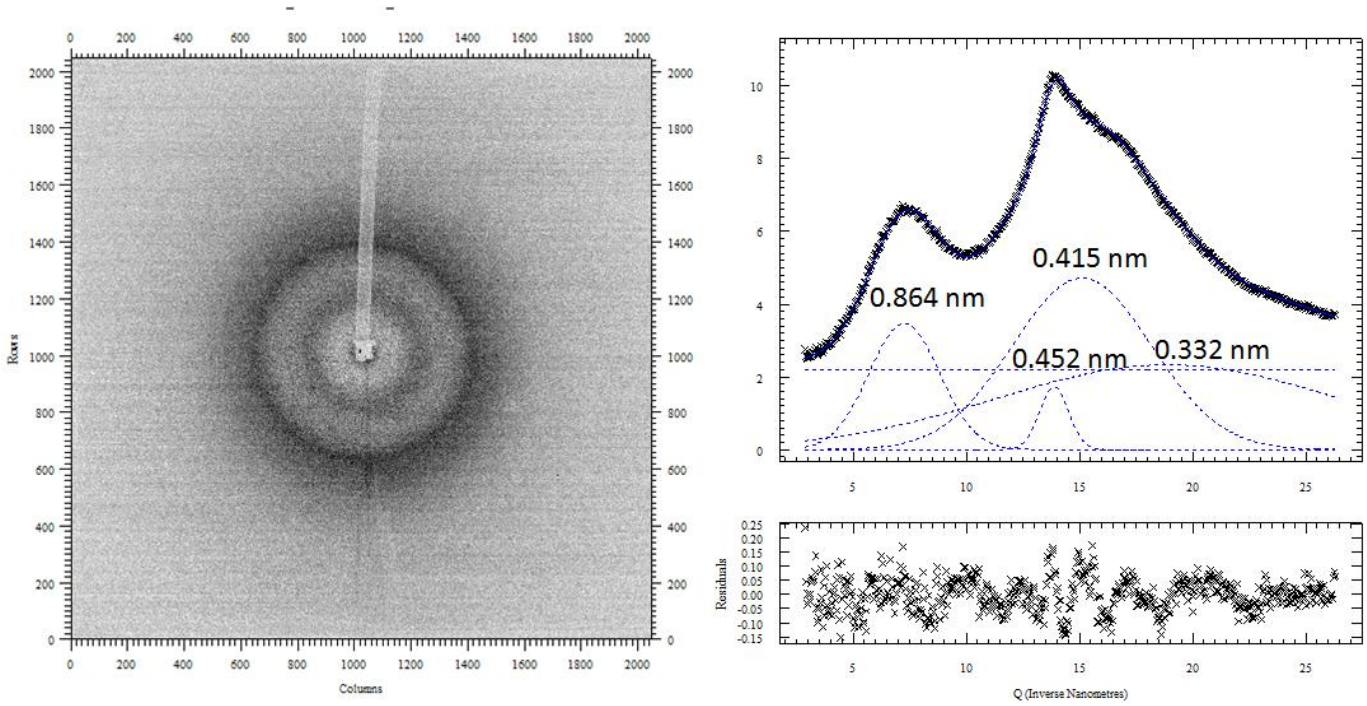

Figure 3. Typical X-ray diffraction pattern (left) coming from the red square region in Figure 2 and related peak fitting (right) obtained with Fit2D.

In figure 3 we show a typical XRD pattern coming from the red-square region of Figure 2 and the related data analysis performed with Fit2D software. We used 3 Gaussian profiles to fit the long range order contributions, a broad Gaussian (d=0.332 nm) for short range order and a 0-order Lorentzian background. The short-range order scattering appears to be random. On the other hand the contemporary presence of the contributions at d=0.864 nm (possible β-sheet stacking) and at d=0.452 nm and d=0.415nm (possible distance between hydrogen-bonded strands) could be attributed to the existence of a β-sheet material. This analysis will need of course further investigation and experimental tests.

In conclusion, answering to the remark of the review committee C07 which commented on our last proposal SC4188 in the following way "Very promising study, but the panel expresses some doubts about the feasibility at ID21. This should be tested before getting beamtime", we showed how the brand new micro-XRD extension of ID21 is suitable for investigating the conformational changes of Alpha-synuclein peptide and in general for probing biosoft matter subjects.

We would like to stress how this beamtime (from 17/6/2015 to 21/6/2015) has been used for XRD just for one day (17/6) while the remaining time was exploited for μFTIR analysis of two different Alpha-synuclein peptides tested at different ranges of temperature and in presence of external agents and nanostructured surfaces.

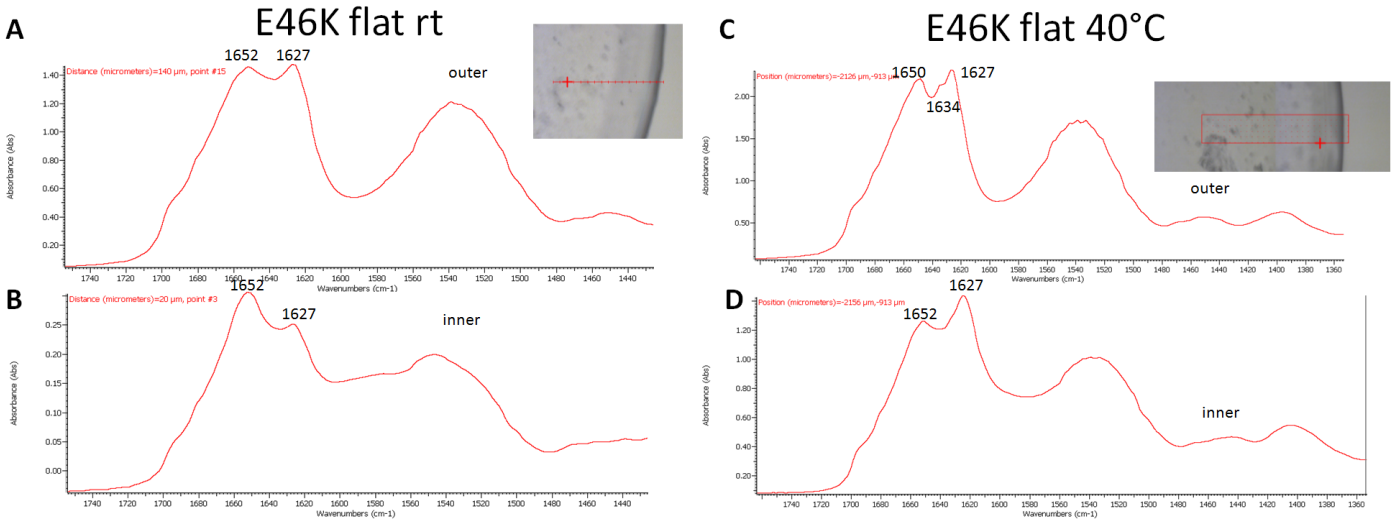

Figure 4. µFTIR analysis of E46K alpha-synuclein peptide at room temperature and at 40°C.

Although the data analysis is still in progress we started to detect some significant conformational changes of the proteins under investigation. As we report in Figure 4A, the E46K alpha-synuclein protein dried at room temperature on hydrophilic supports shows a typical ring-like residue configuration and the coexistence of both β-sheet (1627 cm^{-1}) and α-helical (1652 cm^{-1}) phases in the outer rim. In the inner rim (Figure 4B) the α-helical phase seems to overcome the β-sheet one, probably due to the lower amount of deposited material. On the other hand, by increasing the temperature up to 40°C (Figure 4C,D) we observed that, although the conformation of the outer rim the remains unchanged (Figure 4C), in the inner one (Figure 4D) the β-sheet phase prevails suggesting a possible effect of the temperature which has to be further investigated.

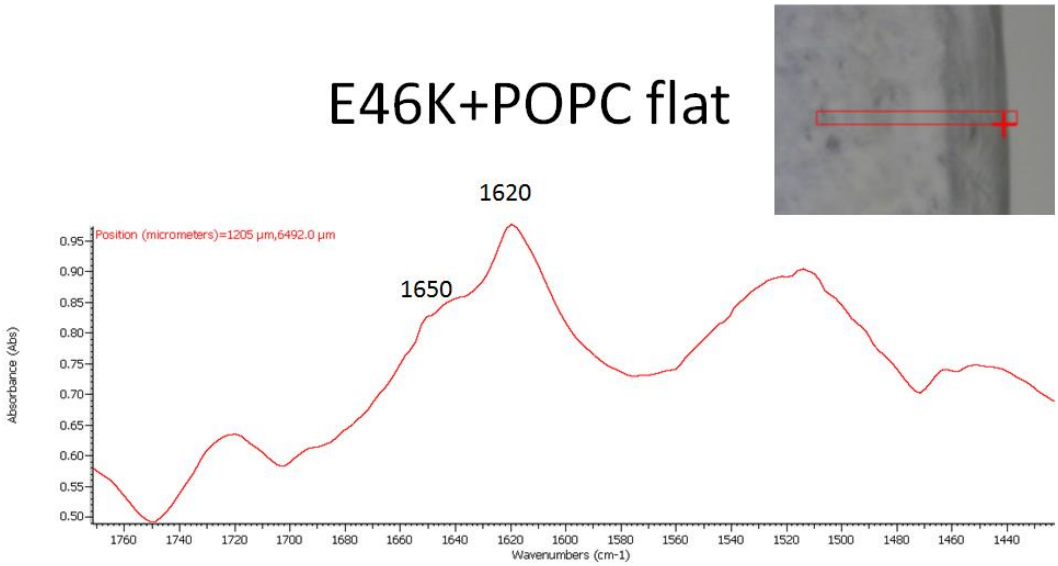

Figure 5. µFTIR analysis of E46K alpha-synuclein peptide in presence of POPC/POPS lipid system.

Finally, by introducing a lipid system POPC/POPS (1-palmitoyl-2-oleoylphospatidylcholine/palmitoyl-2-oleoylphospatidylserine), we observed that the whole E46K alpha-synuclein residue is characterized by a strong parallel β-sheet configuration phase suggesting a possible interaction between the peptide and the lipid vesicles.