Experiment LS-2952 on ID13@ESRF
12 shifts

April 15, 2021@ 08:00 am)
till
April 19, 2022 @ 08:00 am

"Deciphering optical & H+ transport properties in Amyloid fibers: Structure/property correlation through in situ & operando SWAXS under RH, light & ac-electric field stimuli"

Mixed configuration/realization
in-person mode: Dr. M. Marechal and Dr. M. Burghammer)
remote mode: J. Hurtaud (PhD Student), Dr. V. Forge, and Dr. P. Rannou

Non-pathological Amyloid fibers hierachically self-assembled from different proteins, i.e. α-lactalbumin, β-lactoglobulin, lysozyme, and Het-s prion domain, were prepared as described in the proposal LS2952. Two types of samples were studied during the 12 shifts: thin films and membranes made by drop-casting or macrofilaments/macrowires made of aligned unitary fibers (mesoscopic building blocks of ca. 5 to 20 nm in diameter and up to 1 to 20 microns in length) resulting from the evaporative self-assembly of a seating drop (dispersion of amyloid fibers) drying in between two tips (covered by a bee wax). Most of the experiments were performed with a "SAXS" configuration (with a sample-to detector distance of 660 mm). Some samples were additionally characterized with a "WAXS" configuration (sample-to-detector distance of 64 mm). The beam energy was set to 13 keV. Samples were mapped using a 1µm* 2µm shaped beam, with acquisition time of 20 msec. Up to ca. 10,000 2D patterns were recorded for each sample. All experiments were performed in an ambient environment, but without temperature and relative humidity (RH) control. The temperature was 23°C and the RH were 27 ± 1%, staying mostly constant over the 12 shifts. An expected product of the proposal LS2952 was to characterize the samples at different RH values in order to correlate the effect of water on the structure with other physicochemical properties of these amyloid fibers. *Unfortunately, these experiments were only (very) partially successful, a technical problem with the humidity chamber(unfortunately) prevented us to explore a full RH domain (By cycling from 30% till 95% RH and back to 30% RH), critical for progressing toward the determination of the (unsolved till date) structural organization of (other than Het-s-prion) amyloid fibers.*

A truly exceptional set of SAXS/WAXS data could be collected, with a wealth of structural information over the WAXS+SAXS q range, as shown (illustratively) in Figure 1. In addition to the expected "archetypical" Bragg (WAXS) fingerprints for amyloid fibers (4.75 Å and 12.43 Å), several other (unprecedented) Bragg reflections could be detected, most notably in the SAXS region. These data are currently the subject of in-depth analyses

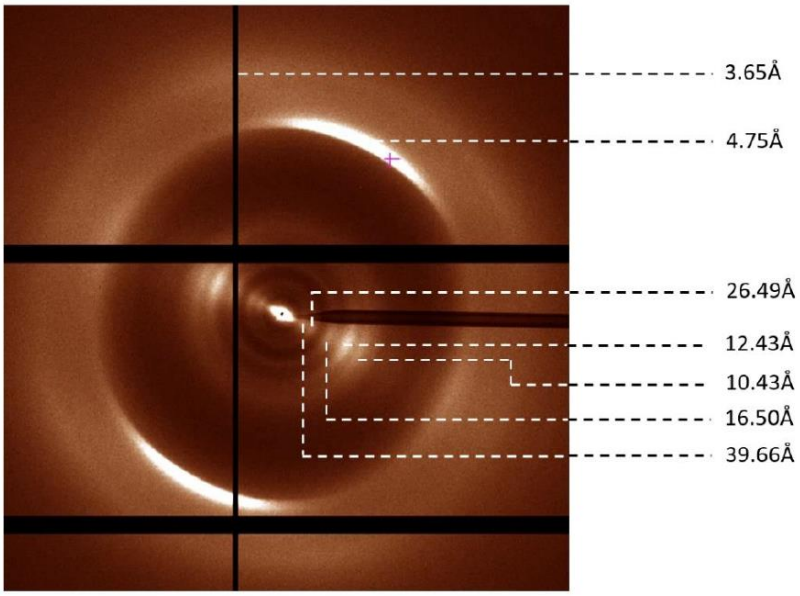

Figure 1: Typical 2D SAXS/WAXS pattern obtained onto α-lactalbumin macrowires.

An Illustrative example of a SAXS/WAXS 2D mapping performed onto *α-lactalbumin macrowires* iss shown in Figure 2. It demonstrates that amyloid fibers (unitary building block) are well-oriented along the macrowire axis, revealing the signature of a hierarchically self-organized structure. More surprisingly, at the extremities of the macrowires, where the amyloid fiber suspension was in contact with the bee wax during the drying process, the fibers are also (very-) well-oriented, but parallel to the contact surface.

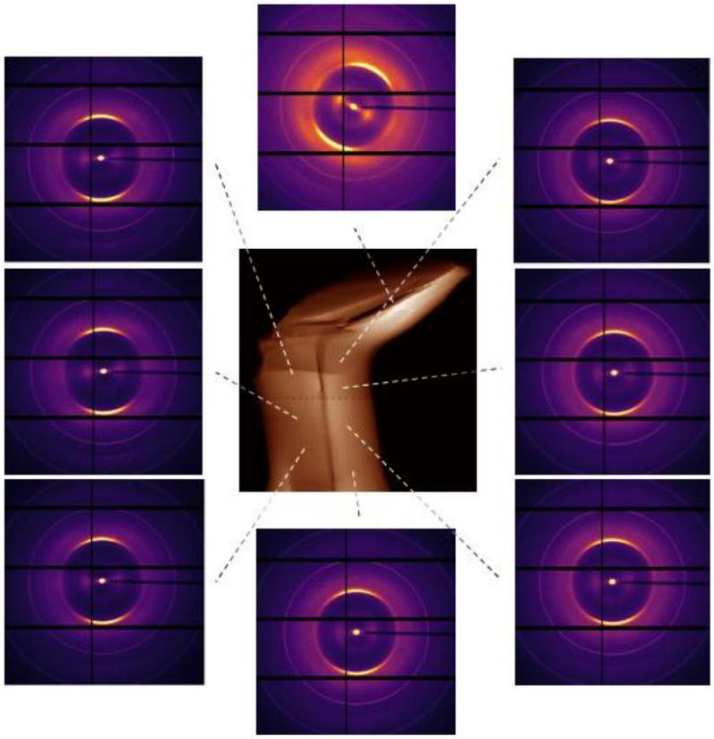

Figure 2: *X-ray mapping with examples of 2D/SAXS/WAXS patterns localized onto an α-lactalbumin macrowire. All 2D patterns are similar in the core of the sample, unraveling a high degree of alignment of the amyloid fibres (the unitary mesoscopic building blocks initially dispersed into a buffer and self-assembled into a microwire) and within the direction of the long axis of the macrowire.*

One strategy currently exploited to extract information contained in the scattering patterns is to define different zones and map their respective intensities in the samples (Figure 3). Zone (3) corresponds to the entire region of the main amyloid component (4.75 Å) (Figure 3; left panel). At the extremities of the macrowire, when comparing two orientations in the amyloid region (3), areas (4) and (5) (Figure 3; right panel), their intensity maxima are not located at the same position, suggesting that several orientations are possible in this part of the macrowire.

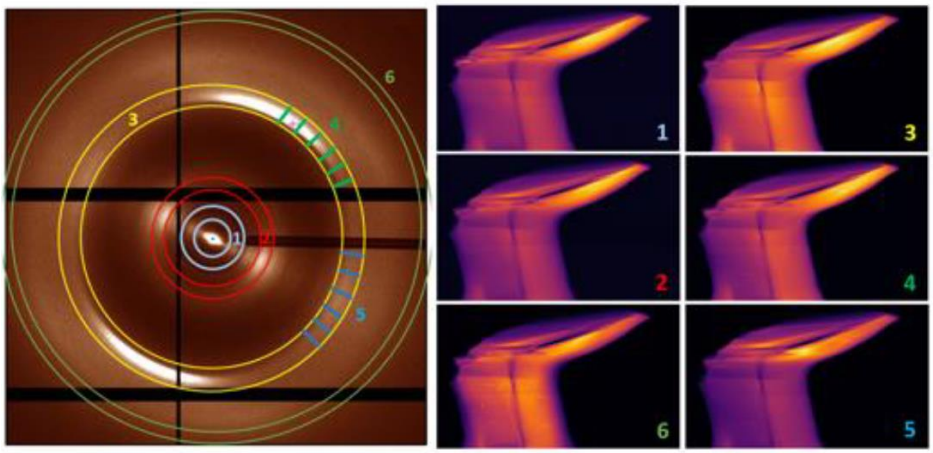

Figure 3: left*) 2D SAXS/WAXS pattern of an α-Lactalbumin macrowire. Six areas are defined for the image reconstruction shown in right panel. (1) & (2) Small angle/(3) Specific amyloid feature at a distance of 4.8Å / (4) & (5) Upper right and lower right zones within (3) respectively. (6) Salts.* ***right****) 2D WAXS maps of the material's core. Each map corresponds to the areas defined in left panel. In the image, each pixel intensity is therefore the result of a specific integration of the 2D SAXS/WAXS apttern.*

Concluding remarks: The experimental time (12 shifts) allocated to the LS-2952 experiments has unfolded the potential of ID13 for the characterization of two types of amyloid fiber samples: i) isotropic thin films (mats of unitary mesoscopic building blocks: not shown here) and ii) hierarchically self-organized macrowires. An exceptional set of data could be collected with a potential wealth of exceptional information, which open doors solving structural organization of amyloid fibers unknown till date. Unfortunately, it was not possible to realize a full dependence of the amyloid fibers structure on RH, which constitute the missing link to fully exploit these very promising set of data. This is justifying (in the view of the co-proposers) the submission (round 10/2022) of the proposal SC-5397 (entitled Leveraging in situ SAXS/WAXS under controlled relative humidity (RH) to understand and control optical and H+ transport properties of amyloid fibers) in the form of a continuation of the LS-2952 experiment