ESRF	**Experiment title:** Langmuir-Blodgett protein multilayers for X-ray diffraction structural analysis by nanofocused synchrotron radiation.	**Experiment number**: **SC-4894**
Beamline: ID13	**Date of experiment**: from: 21 Novembre 2018 to: 24 Novembre 2018	**Date of report**: 25.02.22
Shifts: 9	**Local contact(s)**: Dr Manfred Burghammer	*Received at ESRF:*

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Report:

The main outcomes of the experiment are summarized in the abstracts of following publications (see below). The most prominent results have been obtained with the PGA LB MLs samples, namely, we observe locally globular aggregates and filamentous spherulites based on nanofibrillar subunits with cross-ß amyloidic motifs in the annealed PGA LB MLs. We failed however to perform in-situ heating nanocalorimeric measurements, due to specific geometry of the nanocalorimeter chips that did not allow LB multilayer deposition. Following issues could be addressed in the future experiments:

(1) Deposition of LB-MLs on X-ray transparent nanocalorimetry chips could be used for optimizing in-situ protein nucleation during thermal treatment, as well as allow the temperature modulated nanocalorimetry experiments.

(2) On-line detection of emergent scattering features by mm-scale mesh-scans could be followed up by nm-scale mesh-scans centered on specific scattering features.

(3) Suggested approach could be used for probing new structural features in other protein (e.g. membrane proteins) nanofilms and/or thin films of other biological materials as viruses etc.

1. Pechkova E, Nicolini C., Burghammer M. and Riekel C. Emergence of amyloidic fibrillation in 2D-ordered Langmuir-Blodgett protein multilayers upon heating. *Appl. Phys. Lett.* 2020, 117, 053701.

ABSTRACT

Langmuir–Blodgett protein nanofilms can serve as templates for nucleation and growth of protein crystals. This functionality can be enhanced by thermal annealing. While surface ordering of the multilayered nanofilms and an improvement of the correlation between the layers during thermal annealing have been revealed by atomic force microscopy and grazing-incidence small-angle x-ray scattering, information on the structure developing in the bulk of nanofilms is lacking. In this paper, we report on scanning x-ray nanodiffraction experiments of penicillin-G-acylase multilayers deposited on Si3N4 membranes and annealed at 150 C. While the annealed multilayer has remained mostly featureless, we observe locally globular aggregates and filamentous spherulites based on nanofibrillar subunits with cross-ß amyloidic motifs.

2. Pechkova E, Burghammer M, Nicolini C, Riekel C. 2020. New structural features appear in thermally treated langmuir-blodgett protein multilayers. *NanoWorld J* 6(3): 66-67

SUMMARY

Advanced synchrotron radiation (SR) sources have created many new opportunities for research on the hierarchical structural organization of macromolecular materials. Indeed, micro- and nanobeam GISAXS (grazing incidence small-angle X-ray scattering) techniques were successfully used at the ESRF for probing surface and near-surface organization of 2D – ordered Langmuir-Blodgett protein multilayers (MLs), in particular processes related to thermal annealing. Micro-and nano-GISAXS not only confirm in-plane 2D ordering of MLs, but also reveal an increased correlation between the layers, improving their packing after heating and cooling to room temperature. We are lacking, however, know-how on structural processes in the bulk of MLs which are important for understanding molecular assembly and degradation in ultrathin amorphous protein films. We now have performed 2D raster X-ray nanodiffraction experiments in transmission geometry on penicillin-G-acylase MLs (100 layers) at the ID13 beamline of the ESRF, with an SR monochromatic beam of λ = 0.08157 nm, focused to about 170 x 170 nm 2 spots.

The MLs were deposited on $Si_3 N_4$ membranes with thickness of 500 nm and annealed at 150 ˚C. We were able to record X-ray diffraction patterns from up to several mm^2 areas by an ultrasensitive pixel detector. After heating and cooling, some globular aggregates and filamentous spherulites were observed in PGA MLs by light microscopy, as shown in Figure 1A. Raster X-ray nanodiffraction confirms the emergence of nanofibrillar features with cross-ß amyloidic motifs (Figures 1B and 1D). The spherulite's core structure results in many overlapping filaments with powder-like scattering features. On the spherulites border area, we found instead highly anisotropic scattering, increasing toward the most distant filaments.

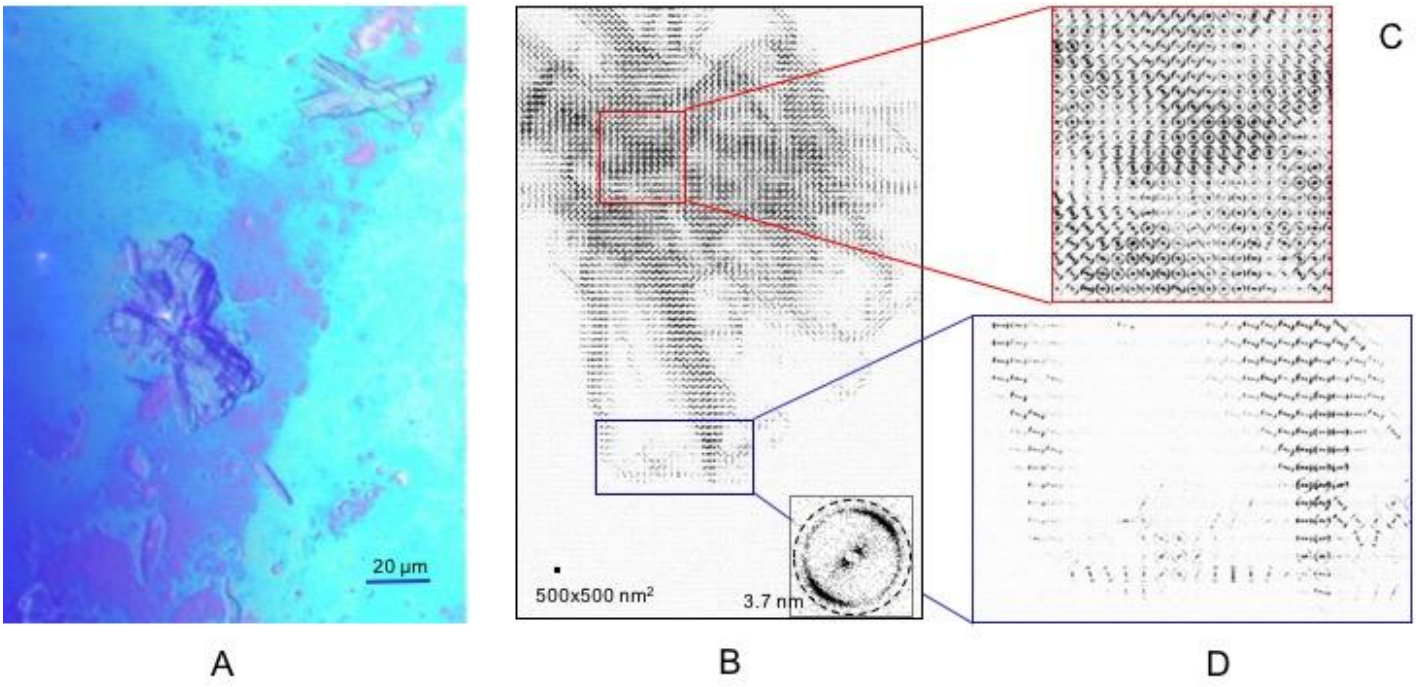

Figure 1: (A) New spherulitic structures appeared in the protein MLs after heating and cooling. (B) The composite of diffraction patterns of a spherulite with 500 nm (hxv) raster step-increments. The resolution range of a pixel is shown in the inset. (C) Zoom of the spherulitic core [red rectangle in (B)]. (D) Zoom of two filamentary arms extending from the core [blue rectangle in (B)].

3. Pechkova E, Nicolini C, Fiordoro S, Riekel C. 2021 published online. Mesoscale ordering of phycocyanin molecules in Langmuir-Blodgett multilayers. *Langmuir* 38 (1):86–91.

ABSTRACT

Phycocyanin molecules, which are part of light-harvesting complexes in cyanobacteria, can be assembled into mesoscale multilayer nanofilms by the Langmuir−Blodgett technique. Results obtained by quartz crystal microbalance and atomic force microscopy confirm the homogeneity and reproducibility of phycocyanin Langmuir−Blodgett multilayer deposition. We show by cryo-electron microdiffraction that amorphous phycocyanin Langmuir−Blodgett multilayers form, after annealing at 150 °C and cooling to room temperature, a layered nanofibrillar lattice with rotational disorder. Scanning X-ray nanodiffraction suggests that structural transformation is not homogeneous through the film but limited to patches of up to about 10 µm diameter.