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New Structural Features Appear in Thermally Treated Langmuir-Blodgett Protein Multilayers

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Advanced synchrotron radiation 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 (LB) 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 [1].

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 nano diffraction experiments in transmission geometry on penicillin-G-acylase MLs (100 layers) at the ID13 beamline of the ESRF [2], with an synchrotron radiation monochromatic beam of λ = 0.08157 nm, focused to about 170 x 170 nm 2 spots. The MLs were deposited on Si3N4 membranes and annealed at 150 °C. We were able to record X-ray diffraction patterns from up to several mm2 areas by an ultrasensitive pixel detector [3].

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 nano diffraction confirms the emergence of nano fibrillar 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.

It will be interesting to compare the results on ML's with other macromolecular materials and in particular performing *in-situ* studies during thermal treatment to study transient phases. Indeed, structural processes associated with high-temperature dehydration of tobacco mosaic virus particle nanofilms have already been studied in-situ by raster X-ray nano diffraction [4].

We used in parallel the possibility provided by ESRF in its Science Building of imaging our samples by atomic force microscopy (AFM). Our aim was to imaging the same MLs sample regions before and after heating to observe changes in PGA MLs surface morphology. AFM imaging was performed at room temperature (22 °C) in tapping mode using an Asylum Research Cypher-S AFM instrument equipped with Bruker MSNL silicon probes. The resonance frequency was measured by the thermal noise method of 107.3 kHz and a spring constant of 0.6 N/m. AFM images were analyzed with Asylum Research software and Gwyddion. The images were acquired with a scan rate from 1.5 to

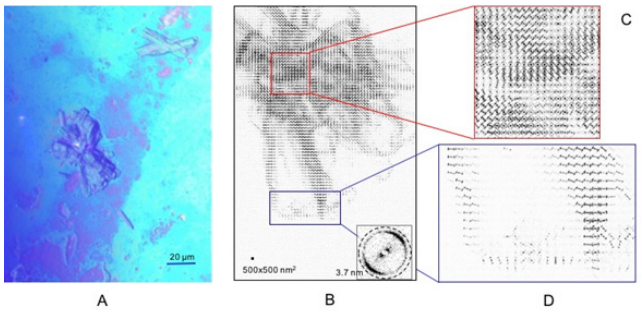

Figure 1: (A) New spherulitic structures appeared in the protein MLs after heating and cooling. (B) The composite of diffraction patterns of a spherulite with 500 nm (hxv) raster step-increments. The resolution range of a pixel is shown in the inset. (C) Zoom of the spherulitic core [red rectangle in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

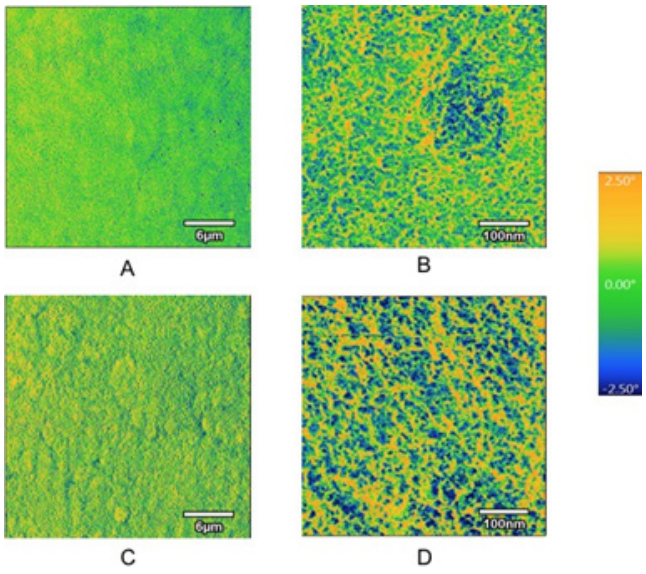

Figure 2: Phase AFM image with two different resolution of PGA LB-MLs (100 layers) before (A, B) and after (C, D) temperature treatment of 150 °C for 10 minutes.

3 Hz. On each sample, several images were produced with a scan size of 6 mm and 100 nm. During scanning, we measured the amplitude and phase signals of the tip oscillation. Figure 2 shows the PGA LB-MLs morphological changes in phase AFM image before and after heating of 150 °C for 10 minutes. Indeed, annealed MLs have a more pronounced pattern in comparison with the unheated sample that remains mostly amorphous.

It is worth to notice that amyloid fibrils play a variety of functional roles in many organisms and have been regarded as potentially useful nanomaterials in recent years, e.g. as templates for bio-mineralization in the synthesis of functional nanomaterials [5, 6]. We suggest that further studies of protein LB multilayers assembly in amyloid motifs upon heating could be useful for the development of new materials macromolecules-based with an advantage of the heat-proof property of LB films including the template for crystallization with the crystalline features, impossible to obtain in protein solution [7].

References

1. Pechkova E, Nicolini C, Burghammer M, Riekel C. 2020. Emergence of amyloidic fibrillation in 2D-ordered Langmuir–Blodgett protein multilayers upon heating. *Appl Phys Lett* 117: 053701. https://doi.org/10.1063/5.0012716
2. Pechkova E, Tripathi S, Nicolini C. 2009. MicroGISAXS of Langmuir–Blodgett protein films: effect of temperature on long- range order. *J Synchrotron Radiat* 16(3): 330-335. https://doi.org/10.1107/S0909049509002763
3. Riekel C, Burghammer M, Dane TG, Ferrero C, Rosenthal M. 2017. Nanoscale structural features in major ampullate spider silk. *Biomacromolecules* 18(1): 231-241. https://doi.org/10.1021/acs.biomac.6b01537
4. Riekel C, Burghammer M, Snigirev I, Rosenthal M. 2018. Microstructural metrology of tobacco mosaic virus nanorods during radial compression and heating. *Soft Matter* 14(2): 194-204. https://doi.org/10.1039/c7sm01332a
5. Wei G, Su Z, Reynolds NP, Arosio P, Hamley IW, et al. 2017. Self-assembling peptide and protein amyloids: from structure to tailored function in nanotechnology. *Chem Soc Rev* 46: 4661-4708. https://doi.org/10.1039/C6CS00542J
6. Janairo JIB, Sakaguchi T, Mine K, Kamada R, Sakaguchi K. 2018. Synergic strategies for the enhanced self-assembly of biomineralization peptides for the synthesis of functional nanomaterials. *Protein Pept Lett* 25(1): 4-14. https://doi.org/10.2174/0929866525666171214110206
7. Pechkova E, Nicolini C. 2017. Langmuir-Blodgett nanotemplates for protein crystallography. *Nat Protoc* 12(12): 2570-2589.