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Meeting Abstracts

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Featured Presentations

Langmuir-Blodgett Thin Films and Cancer Proteomics

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Abstract

Langmuir-Blodgett (LB) thin films have been used as molecular bio-templates for facilitating macromolecular and protein crystallization, an approach pioneered by Pechkova and Nicolini in the early 2000s. LB-mediated crystallization has been found to lead to remarkable changes in protein stability and water dehydration, despite slight/negligible changes in protein atomic structure. We have previously discussed the importance of LB-based nanocrystallography at the frontiers of cancer proteomics focusing on two model proteins with important biological roles in cancer, which have been extensively studied and modeled, for investigating protein folding, disulfide bond formation, and protein dynamics, using protein crystallography or spectroscopy. These protein models are CK2alpha and RNase A. In particular, computational mutagenesis using the KINARI Mutagen web-server has shown to exhibit different behaviors in terms of protein stability and robustness, as well as in terms of water dynamics. Introduction of LB film seems to lead to the appearance of water molecules close to the protein surface with larger volume, causing changes in crystal stability, protective against radiation and appearing replicated in mutant proteins. Implications for drug design, drug delivery and cancer-causing protein variants are herein envisaged and discussed, along with a review of the most recent findings in LB-based nanobiocrystallography and with an overview of future prospects, including last developments and achievements in fabricating long-lasting drug delivery tools and spatiotemporally and sequentially-controlled drug releasing devices (the so-called "chronotherapeutics").

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Biography

Nicola Luigi Bragazzi was born on the 2nd of March in 1986 in Carrara (MS), Tuscany (Italy) and is currently a MD, a PhD and a specialist in Public Health. He got his MD (medical degree) on the 15th of July in 2011 with a final mark of 110/110 cum laude with a thesis on Personalized Nanomedicine ("Nanomolecular aspects of medicine, at the cutting edge of the nanobiosciences in the field of health-care") discussed with Professor Claudio Nicolini, Eugenia Pechkova and Victor Sivozhelezov. He got his PhD in biophysics at Marburg University, Germany with a final mark of "very good", with a thesis discussed with Professors Claudio Nicolini, Norbert Hampp and Eugenia Pechkova. Moreover, he has been awarded as Young Knight of the Italian Republic by the President Carlo Azeglio Ciampi and is an Overseas Fellow of the Royal Society of Medicine, one of the major and most prestigious providers of accredited postgraduate medical education in the United Kingdom.

Protein LB Crystal via NIMA vs Protective Plate

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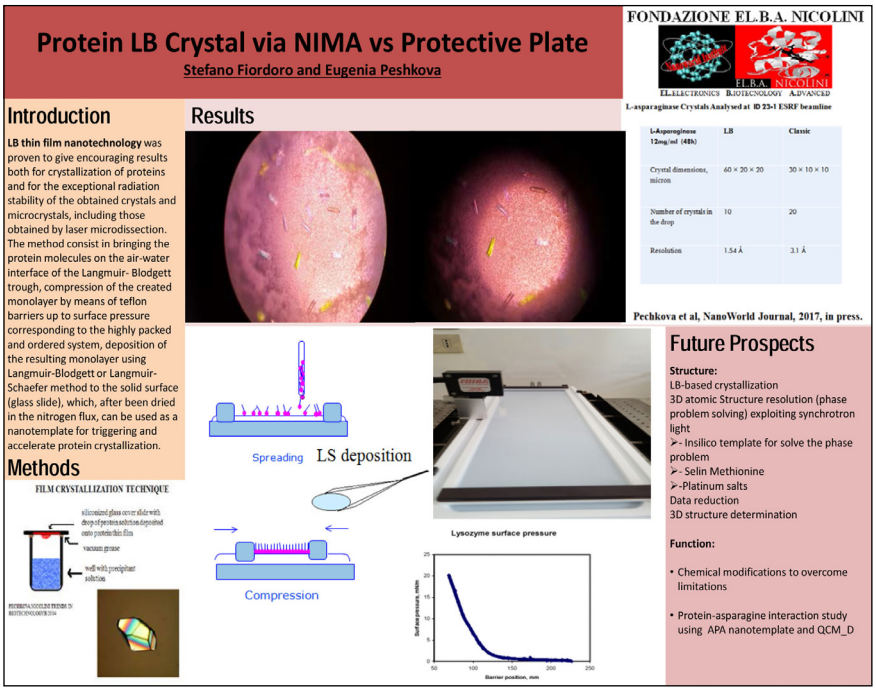
Abstract

LB thin film nanotechnology was proven to give encouraging results both for crystallization of proteins and for the exceptional radiation stability of the obtained crystals and microcrystals, including those obtained by laser microdissection. The method consist in bringing the protein molecules on the air-water interface of the Langmuir-Blodgett trough, compression of the created monolayer by means of teflon barriers up to surface pressure corresponding to the highly packed and ordered system, deposition of the resulting monolayer using Langmuir-Blodgett or Langmuir-Schaefer method to the solid surface (glass slide), which, after been dried in the nitrogen flux, can be used as a nanotemplate for triggering and accelerate protein crystallization.



Crystal Stability by Means of Molecular Dynamics (MD) and Monte Carlo (MC) Simulations

Philipp S. Orekhov

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Abstract

Modern X-ray crystallography is a dominant method for structure determination of large biomacromolecules such as proteins, nucleic acids and their complexes. It allows to reconstruct atomic structure of biomacromolecular specimens with the atomic weights up to millions Da and with the resolution down to 1 Å, especially after appearance of such modern and extremely intense X-ray radiation sources as free electron lasers (FELs). However, crystal stability and damage due to interactions of matter with the X-ray radiation limits time of diffraction data deposition and, thus, the resolution of the obtained electron

density maps, which serve as a starting point for determination of the atomic positions. It was previously shown practically and theoretically that the Langmuir-Blodgett (LB) crystals have higher stability against radiation damage in comparison with the classical hanging drop (HD) crystals similarly to the space-grown (SG) crystals. However, it remains still unclear how and to what extend the methods utilized for the crystal growing and the micrometer- and nanometer- scale structure of crystals influence the crystal stability particularly against the radiation damage. In the present study we address the problem of crystal stability by means of molecular dynamics (MD) and Monte Carlo (MC) simulations.

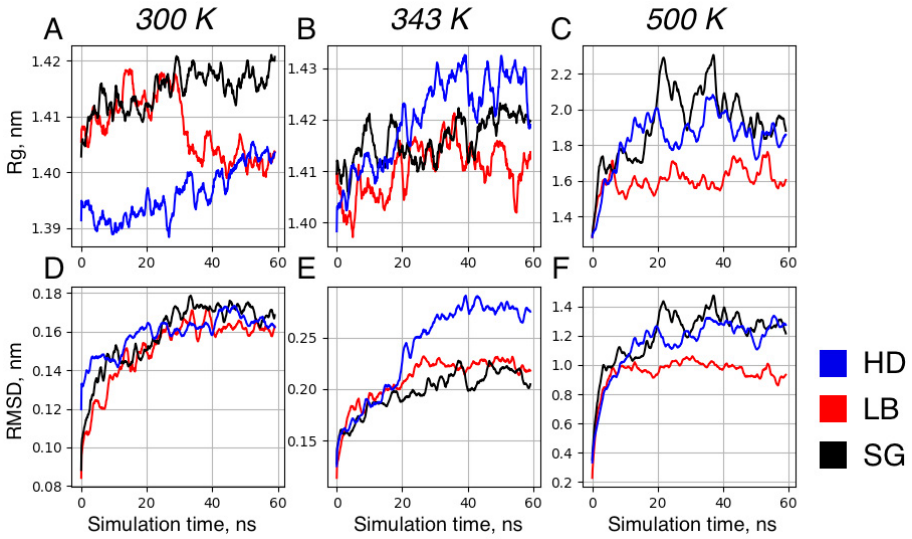

Figure 1: (**A-C**) Gyration radius averaged over 16 individual proteins in the crystal unit for the three systems (HD – hanging drop, LB – Langmuir-Blodgett and SG – space-grown) simulated at three different temperature values (300, 343 and 500 K) plotted as a function of the simulation time; (**D-F**) RMSD averaged over 16 individual proteins in the crystal unit for the three systems (HD, LB and SG) simulated at three different temperature values (300, 343 and 500 K) plotted as a function of the simulation time.

The simulations indicate that at the elevated temperatures the space-grown and LB crystals are more stable than the classical HD one (see Figure 1). The observed thermostability of the LB and SG crystals is likely due to the higher content of the mobile water molecules, which can serve as a heat bath, which takes an excessive heat from proteins and, at the same time, split them apart more efficiently.

At the same time, combining the molecular dynamics simulations with the Monte Carlo approach for estimation of the ionization events number, we have shown that the LB crystals are also more tolerate to the electrostatical effects of ionization comparing to the classical HD crystals.

Biography

Dr. Philipp S. Orekhov received his B.S. and M.S. in Biophysics from M.V. Lomonosov Moscow State University in 2010 and his Ph.D. from the Osnabrueck University in 2016. He is currently employed in Moscow Institute of Physics and Technology and his research interests include molecular simulations of large protein complexes including membrane proteins, interactions of the artificial nanoparticles with biomembranes and combination of molecular simulations with bioinformatical approaches for analysis of protein structure and dynamics.

Confinement of Lysozime in Anodic Porous Alumina

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Abstract

Anodic porous alumina was considered as an inorganic scaffold, which found futuristic used for the confinement of protein, nucleotide sequences, and gene expression [1, 2]. The confinement of protein in a matrix of anodic porous alumina has been considered as a primary point of interest due to the remarkable properties of compatibility with human cell and due to high order disposition in a periodic array. Following the ordinary definition of the array, it was defined as a periodic sequence with

pores periodicity in the structure able to maintain multi bio-spot, which due to high transparency can be used as multi-spot arrays to investigate the protein-protein interaction from blood serum or protein-gene interactions. The potentiality of these high-density pores for centimes squares up to 108 pores/cm^2 revert a factorable interest to minimize false positive data set in procedures of protein interaction. This request is particularly of interest for clinical use due to remarkable influenced of this false positive in the statistical analysis of data. In this Master thesis, Camera Coupled Device use CCD camera, was employed potentially as possible microscopy instrument to study in real-time the protein-protein interaction between protein and protein using cyanine dye molecule as probes. Moreover, this is possible because of the anodic porous alumina dimensions are within the zone of microscopy magnification [3]. APA achieved from current literature review a range between 10 to 500 nm [4], obtained by electrochemical routes. In our procedure, oxalic acid in electrolytic solution was employed to achieve our goal. This restricts conditions of dimensions have limited its use due to not solved confinement in a reduced area of nano-micro channels. As we know, during the growing of anodic porous alumina, there is an aluminum redistribution of mass, density, and porosity; the stressed on the material strictly have an influence on the growth phase of pores and on its dimensions. During the preparation by the electrolytic route the physical stress of the material plays a role onto the definition of pore order and also on its shape. In our case, the stress induced on the aluminum foil is the key to obtaining poses in micro-scale dimensions. These key solution offers not only the opportunity to use anodic porous alumina as a matrix to be observed during the confinement evaluation by CCD camera but open the prospect of a new easy low cost and visible confinements. Moreover, reduced scale dimension in micro zone in anodic porous alumina solved a tricky of capillary force in the common one is used in Nano scale dimension. The transparency of anodic porous alumina with micro dimensions of pores offers as a scaffold for a major volume of confinement to be monitored by absorption spectroscopy. Lysozyme was proposed as a protein of reference as the low molecular weight has been dropped by solution casting on a matrix of anodic porous alumina. Spectra absorption confirms the amount of protein, but also the transparency of the membrane. The mouldable change the pore dimension and the thickness of the membrane has significantly influenced the aliquot of distributions and the interference of matrix with absorption band in Spectroscopy. In the course of this Master, thesis was tired also as a possible utilized of anodic porous alumina used for the biofilm layer in the Langmuir-Blodgett film. CCD camera image acquires several frame images at different acquisition time. This choice discriminate the native fluorescence of APA from the protein-marked sample. In Figure 1 we reported the native fluorescence of Anodic porous alumina and the fluorescence of anodic porous alumina after the confinement of Lysozime.

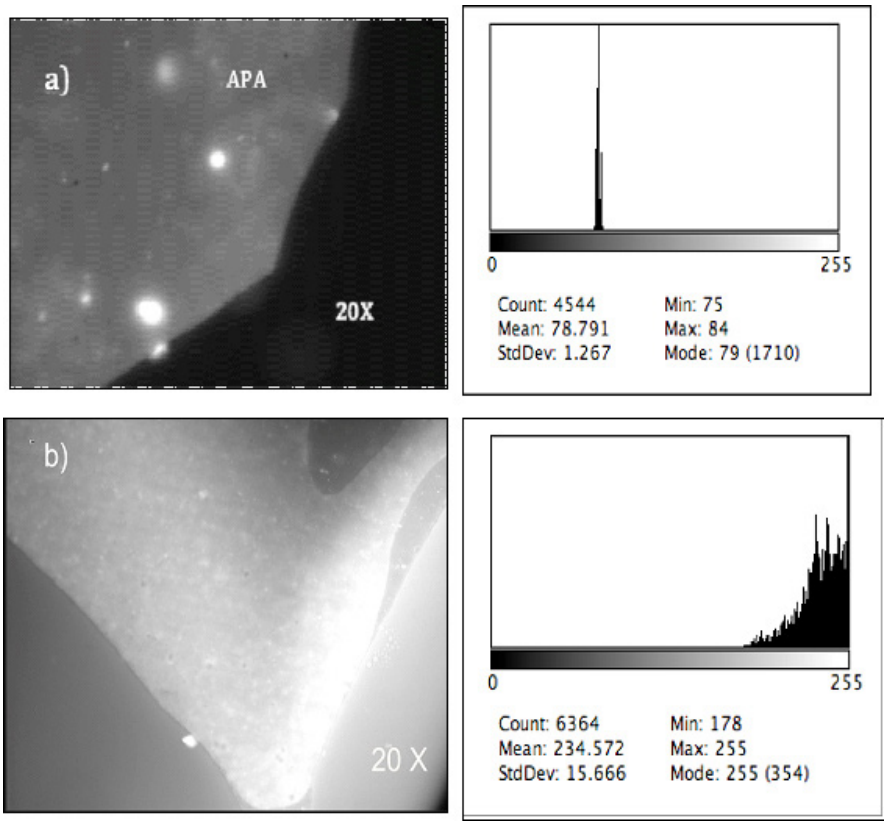

Figure 1: **(A)** Normal fluorescence of anodic porous alumina. **(B)** Fluorescence of anodic porous alumina after the Lysozyme confinement.

Note: Scale of grey from 0 to 250 has been assumed

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Table 1: Report of activities.

Dr. Claudio Larosa	Date (year)	Average Pore Dimensions	Average Pores Depth
Ph.D	2008	1.5 µ	1.5 µ
Latvia (stage)	2016	1.2 µ	700 nm
Master activities	2015/2017	60 nm ± 10	500 µ

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