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Meeting Abstracts

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Proceedings ELBA NW Nanoforum XLII. Part-3: Nanotechnology for Structural Proteomics and Conclusions

NANOTECHNOLOGY FOR STRUCTURAL PROTEOMICS

Nanotechnology, fundamental in achieving the previously described progress in X-ray free electron lasers and synchrotron radiation. is essential in yielding significant progress in the resolution of the crystallization problems and of protein crystallography by cell free expression nanoarrays, polymers, enzymes, nanoconductimetry, mass spectrometry, APA and Monte Carlo Simulation, and by the development of highly ordered 2D and 3D protein LB templates. Particularly relevant to the resolution of pending dramatic problems of humanity are also the advances in Solar Energy and Molecular Bioelectronics induced by Nanotechnology applications to Structural Proteomics.

Claudio Nicolini, President Fondazione ELBA Nicolini and NanoWorld HighTech LLC, USA

Unique Method Based on Langmuir-Blodgett Nanotemplate and GISAXS for the Solution of the Open Problems in Protein Crystallography

Evgeniya Peshkova

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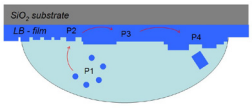
Abstract

With the new generation of synchrotrons and microfocussed beamlines a great progress could be achieved in the area of X-ray protein crystallography resulting in new protein 3D atomic structures of high interest to pharmaceutical industry and life sciences. However, the production of the protein crystal as well as its quality (order, intensity of diffraction, radiation stability) remain open problems. This protocol introduces Langmuir-Blodgett (LB) nanotemplate method, applicable to any protein (including membrane proteins). Highly ordered 2D LB thin protein films preparation on the air-water interface and their deposition on the glass slides are described in details as well as application of these films as a 2D template for triggering of 3D protein crystals in the hanging drop vapor diffusion. The procedure takes few minutes. The structural information about the reorganization in the LB film during crystallization process on the nano level can be obtained by in situ sub-micron GISAXS (Grazing Incident Small Angle X-ray Scattering) method. MicroGISAX spectra, measured directly on the interface of the LB films and protein solution in real time, can be interpreted in terms of the buildup of layers, islands or holes. These data give new insights to the described phenomena of LB nanotemplate and its ability to trigger and accelerate protein crystallization. In comparison to the standard crystallization protocols, the obtained crystals are more ordered and radiation stable. The development of highly ordered 2D protein LB templates can approach the resolution of the crystallization problem, also with the recent progress in X-ray free electron lasers and cryo-electron microscopy.

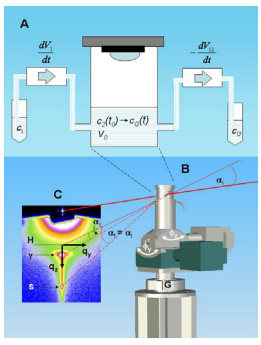
MODEL for reaction pathways on LB-film



Setup of *in situ* μGISAXS at ID13 beamline/ESRF. The flow through crystallization cell is tilted by ϕy to adjust a fixed angle of incidence (αi). As typical features the Yoneda Peak (Y) at α_c and the specular peak at α_f=αi are shown in the 2d GISAXS pattern.

Gebhardt, Pechkova, Riekel, Nicolini, Biophysical Journal, Part II, ***99, 2010, 1262–1267***

Pechkova, Gebhardt, Riekel, Nicolini, Biophysical Journal, Part I, ***99, 2010, 1256–1261***



References

1. Pechkova E, Tropiano G, Riekel C, Nicolini C. 2004. Radiation stability induced in protein crystals grown by nanobiofilm template technology: synchotron microfocus analysis. *Spectrochim Acta B* 59: 1687-1693.
2. Pechkova E, Nicolini C. 2003. Proteomics and Nanocrystallography. Kluwer Academic/Plenum Publishers, New York, USA.
3. Pechkova E, Nicolini C. 2004. Protein nanocrystallography: a new approach to structural proteomics. *Trends Biotechnol* 22: 117-122. https://doi.org/10.1016/j.tibtech.2004.01.011
4. Pechkova E, Tripathi S, Nicolini C. 2009. MicroGISAXS of Langmuir-Blodgett protein films: effect of temperature on long-range order. *J Synchrotron Radiat* 16(Pt3): 330-335. https://doi.org/10.1107/S0909049509002763
5. Pechkova E, Tripathi S, Ravelli R, McSweeney S, Nicolini C. 2009. Radiation stability of proteinase K grown by LB nanotemplate method. *J Struct Biol* 168: 409-418. https://doi.org/10.1016/j.jsb.2009.08.005
6. Pechkova E, Gebhardt R, Riekel C, Nicolini C. 2010. *In situ* GISAXS: I. Experimental setup for submicron study of protein nucleation and growth. *Biophysical Journal* 99(4): 1256-1261. https://doi.org/10.1016/j.bpj.2010.03.069
7. Nicolini C, Belmonte L, Riekel C, Koenig C, Pechkova E. 2014. LB nanotemplate crystallization combined to laser-microfragmentation uniquely characterize proteins crystals by synchrotron microdiffraction. *Am J Biochem Biotechnol* 10: 22-30.
8. Nicolini C, Wright J, Pechkova E. 2015. Synchrotron diffraction of multilayered LS PGA film after heating and cooling. *NanoWorld J* 1(1): 4-8. https://doi.org/10.17756/nwj.2015-001
9. Pechkova E, Nicolini C. 2017. Unique method based on LB nanotemplate and GISAX for the solution of the opern problems in protein crystallography. *Nature Protocols*. (Accepted, In Press)

Biography

After taking her Doctoral degree in Chemistry at Moscow State Lomonosov University, Russia in 1998 and PhD degree in Biophysics with the thesis entitled "Protein Crystallography By Thin Film Nanotechnology" in University of Genova, Italy, following by a PostDoc position, in 2003 became a Scientific Secretary and in 2006 a Scientific Director of Fondazione EL.B.A. (Electronic Biotechnology Advanced), Rome, Italy, being a Principle Investigator of a big FIRB research grant on Organic Nanotechnologies and Nanosciences. In 2007 she worked as a Visiting Scientist at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France both in Macromolecular crystallography, Soft condensed matter and Micro/Nanofocus group. From 2008 she has the permanent position of Assistant Professor of Biochemistry at the University of Genova Medical School with national habilitation to Associate Professor from July, 1, 2014, heading the Laboratories of Biophysics and Nanotechnology. She carried out stages at Harvard University; IBM at Almaden, Jefferson Cancer Center, University of Massachusetts Medical School, UCLA and ASU. Author of 84 international scientific publications (SCI), 2 patents; 15 chapters to books, a textbook on "Proteomics and Nanocrystallography", the volume "Synchrotron Radiation and Structural Proteomics". H-index 21; 50 protein structures deposited in PDB data bank.

Solar Light Harvesting, a New Biological Structure in Between a Reaction Center and a Photosystem

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Abstract

In the focus of photosynthetic reactions is the process of converting the light energy into chemical energy this process is maintained by protein pigment complexes which are called reaction centers(RC). The importance of these RC's can be seen as fundamental for any photosynthetic process. The first ever solved membrane protein structure was of the bacterial reaction center which led to the thesis that the oxygen evolving photosystem II is in its reaction center structurally closely related. In the year 2001 the first structure of photosystem II and the high resolution structure of photosystem I proofed this hypothesis. The reaction center of *H. modesticaldum* takes a special place in between the other known structures. The *H. modesticaldum* reaction center is not an ancestor of any known reaction center but gives input to the evolution of photosynthesis. From the chemical nature of the Chlorophylls (three different types) as its orientation in the core of the reaction center as its adjacent integrated Chlorophyll based antenna system it is in size and composition unique. Distances between Chlorophylls in the reaction center P800 compared to P700 and to the terminal acceptor FX are different to photosystem I. In the high resolution structure of *H. modesticaldum* reaction center the farnesyl side chains can be assigned from electron density as the nature of the over 50 Chlorophylls in the homodimer. (Funding support ongoing since 2013 by DOE grant DE-SC0010575).

Biography

The inner sanctum of photosynthesis ----the structure and function of Photosystem I and II-- has been the focus of my research interests since my master thesis and Ph.D. For the past decade, I have had the opportunity to work on crystal structures of various proteins in the broad field of photosynthesis. Membrane proteins are the most interesting and challenging proteins of all. Currently, the Protein Data Bank (PDB) has more than 125,000 structures. In contrast, the number of known membrane protein structures is still around 600 unique structures. Therefore the majority of the most important proteins are still unknown by their structure. The field of membrane protein structure determination is still in the beginning with a clear growing impact to many research topics in chemistry, biochemistry, biology and medicine. Dr. Fromme has contributed to over 50 PDB structures including 7 membrane protein structures, the results are published in over 50 mostly high ranking articles.

Protein Crystals Studied by Means of Monte Carlo Method

P.S. Orekhov and Claudio Nicolini

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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.

Thermal Stability of Lysozyme Crystals Obtained by Different Techniques Investigated Using MD Simulations

For MD simulations we have chosen three structures of lysozyme representing different crystallization techniques (Table 1): classical "hanging drop" (HD), Langmuir-Blodgett (LB) and the space-grown in microgravity conditions (SG). The crystal triclinic supercell was set up for all models. Each cell contained 16 proteins, the total charge of such system was +128. The cells were solvated with the TIP3P water model and the chlorine ions were added in order to neutralize the systems.

Table 1: Data about the simulated systems.

PDB ID	Unit cell sixe nmxnmxnm	Resolution nm	Volume Nm3
	7,65´7,65´3,61	0,17	481
	7,92´7,92´3,74	0,17	564
	7,71´7,71´3,72	0.094	541

All simulations have been conducted in GROMACS 2016 package using the CHARMM36 full-atom force field. Equilibrium simulations were run for 100 ns followed by the production simulations each 60 ns long at three temperatures – 300 (optimal conditions for lysozyme functioning), 343 (a bit lower than the thermal denaturation T for lysozyme) and 500 K (sufficiently above the thermal denaturation T). The structural parameters of all three crystals (Figure 1) simulated at 300 K are similar and stable with the RMSD values fluctuating around 1.6-1.8 Å and the gyration radius around 1.40-1.41 nm. However, already at this low temperature the volume and the crystal density differ significantly between the systems what appears as an inherited property of the used crystal structures (Table 1): the HD crystal has the lowest volume and the highest density, while for the LB it is vice versa.

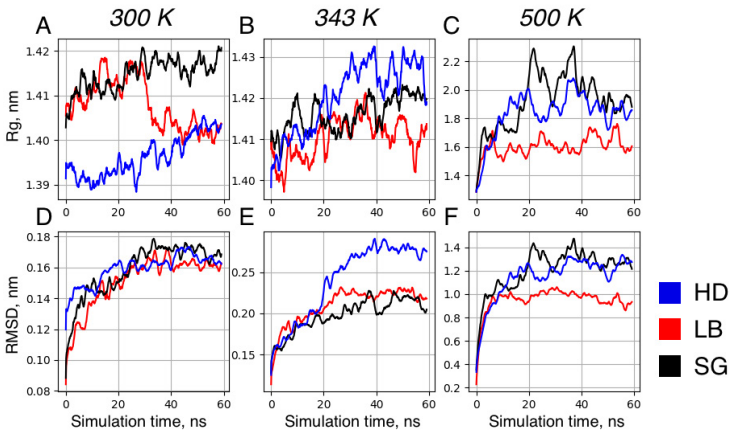

Figure 1: (**A-C**) Gyration radius averaged over 16 individual proteins in the crystal unit for the three systems (HD, LB and SG) simulated at three different temperature values (300, 343 and 500 K) plotted as a function of the simulation time; (**D-F**) RMSD averaged over 16 individual proteins in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

With the increase of the temperature the discrepancy in the stability of the three crystals emerge, both at the pre-denaturation temperature (343 K) and post-denaturation temperature (500 K). The LB crystal structure appears as the most stable in the all of the performed simulations.

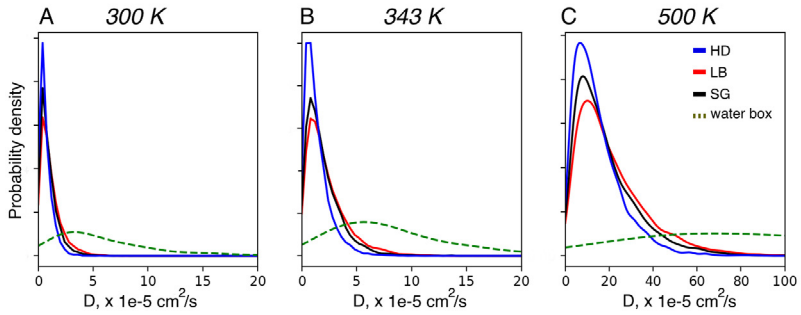

Figure 2: Distributions of the water diffusion coefficient calculated for the three systems (HD, LB and SG) simulated at three different temperature values (300 (**A**), 343 (**B**) and 500 (**C**) K) along with the distributions of the water diffusion coefficient calculated from the short simulation of the pure water box (10 ns) at the corresponding temperature values.

Since one of the major difference between the investigated crystal models is the water content of them, we have investigated the water mobility in terms of the diffusion coefficient, *Ddiff*, in all three systems at three different temperatures. We have found out that in the LB and SG systems there is a noticeable shoulder at the higher *Ddiff* values. Presumably, the main peak of the *Ddiff* distribution corresponds to the "slow" water molecules bound to proteins, while in the crystals with the bulkier unit cells (i.e., LB and SG) there appear an additional fraction of more mobile water molecules with the *Ddiff* similar to the bulk water (compare solid curves to the dashed one on Figure 2). These additional water molecular likely serve as a thermal bath, which draws heat from the protein molecules and acts against the structural collapse. With the increase of the temperature the discrepancy in the stability of the three crystals emerge, both at the pre-denaturation temperature (343 K) and post-denaturation temperature (500 K). The LB crystal structure appears as the most stable in the all of the performed simulations.

Stability of the Ionized Lysozyme Crystals

Using a similar computational protocol, we have investigated the effects of ionization on stability of the LB and HD crystals. Ionization was simulated as a random assignment of the +1 charge. The fraction of the ionized atoms was estimated using the Monte Carlo simulations in the Geant4 program and equaled 1/100 and 1/1000 of the total atom number. The MD simulations were performed at 100 K for 10 ns. While no significant difference was observed in the simulations with the 1/1000 ionization fraction, when 1/100 of atoms were ionized we found a minor dissimilarity between the LB and HD systems in terms of the RMSD and the RMS fluctuations per residue (Figure 3). The LB crystal model appeared more stable in these simulations.

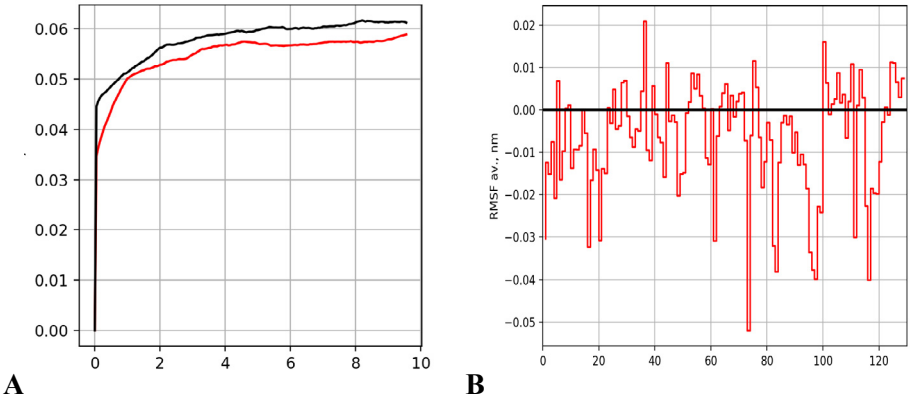

Figure 3: (**A**) RMSD of the LB (red) and HD (black) systems as a function of the simulation time. (**B**) The RMS fluctuation difference between the LB and HD systems.

At the pre-denaturation and post-denaturation temperatures the spacegrown and LB crystals are more stable than the classical HD one; 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 and split proteins apart more efficiently; The LB crystals are also more tolerate to the electrostatical effects of ionization comparing to the classical HD crystals.

Strutural NanoProteomics by Cell Free Expression, Polymers, Enzymes, Nanoconductimetry, Mass Spectrometry, APA, LB and Montecarlo

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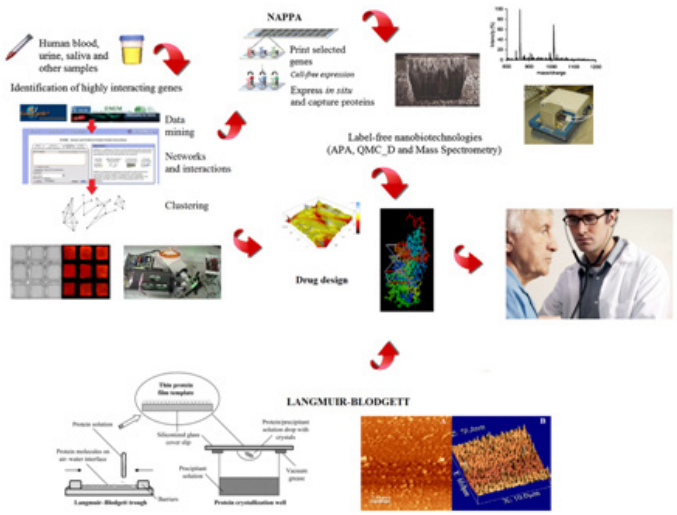
Abstract

A review of the emerging trends and perspectives of Protein Nanocrystallography is presented at the conclusion of this Nanoforum at the intersection of advances in nanotechnology (Langmuir-Blodgett and Anodic Porous Allumina), proteomics (microarray, cell free expression and SNAP) and synchrotron radiation (third generation sources trillion times more brilliant requiring quite smaller crystals and Monte Carlo simulation presented in the previous paper). It should be noted that nanocrystallography here does not refer to crystals of nanometer size or to nanodrop crystallization technology, but to the significant applications for medicine emerging in our labs at the interface of Langmuir-Blodgett engineering, organic chemistry, molecular dynamics and label-free Protein Arrays, utilizing bacterial hell's gate globin, octopus rhodopsin, bovine cytochrome, human kinase, laccase and many other proteins.

A review of the emerging trends and perspectives of Structural Proteomics is presented in the Figure below at the intersection of advances in nanotechnology (Langmuir-Blodgett and Anodic Porous Allumina), proteomics (microarray, cell free expression and SNAP, Mass Spectrometry, Nanoconductimetry), synchrotron radiation (third generation sources trillion times more brilliant using quite small crystals and Monte Carlo simulation) up to its clinical applications to humans (1) and to industrial applications (2) in hardware and energy. It should be noted that here we introduced nanocrystallography not referring to crystals of nanometer size or to nanodrops crystallization technology, but to the significant applications for medicine emerging in our labs at the interface of Langmuir-Blodgett and APA engineering, organic chemistry, recombinant DNA, molecular dynamics and label-free Protein Arrays, utilizing bacterial hell's gate globin, octopus rhodopsin, bovine cytochrome, human kinase, laccase and many other proteins important for health (1) and new hardware (2). All the above industrial and clinical applications will be carried out in the United States by a new company NanoWorld High Tech LLC USA (recently create by myself in Boston, Massachusetts), in close cooperation with companies located in the Boston and Tempe areas and with the Fondazione ELBA Nicolini proprietary of many relevant patents acquired in the activities described in the introduction of this Proceeding and in my biography below.



References

1. Nicolini C, Bragazzi NL, Pechkova E. 2016. Microarray-based functional nanoproteomics for an industrial approach to cancer: II bioinformatics and miRNAome. *NanoWorld J* 2(1): 1-4. https://doi.org/10.17756/nwj.2016-019
2. Nicolini C, Bragazzi NL, Pechkova E. 2016. Microarray-based functional nanoproteomics for an industrial approach to cancer. II mass spectrometry and nanoconductimetry. *NanoWorld J* 1(4): 128-132. https://doi.org/10.17756/nwj.2016-017
3. Nicolini C, Bragazzi NL, Pechkova E. 2016. Label-free mass-spectrometry (MS) protocol. *Nature Protocol Exchange*. https://doi.org/10.1038/protex.2016.002
4. Nicolini C, Bragazzi NL, Pechkova E. 2016. Quartz crystal micro-balance with dissipation factor monitoring (QCM_D) protocol. *Nature Protocol Exchange*. https://doi.org/10.1038/protex.2016.003
5. Nicolini C, Bozdaganyan M, Bragazzi NL, Orekhov P, Pechkova E. 2016. Molecular dynamics (MS) protocol. *Nature Protocol Exchange.* https://doi.org/10.1038/protex.2016.016
6. Nicolini C. 2016. Molecular Bioelectronics – The 19 years of Progress. Second Edition. World Scientific, Singapore, USA, and UK, pp: 1-315.

Biography

Born in Udine, after taking his Doctoral Degree in Nuclear Physics at the University of Padua, became firstly Assistant Professor and subsequently Researcher Collaborator at the National Institute of Nuclear Physics. From 1968, except for a short period (1970-1971), in which he was Adjunct Professor of Physics at the University of Bari, Claudio Nicolini has been living for 16 years in the U.S.A. (the citizenship of which he still has) first as nuclear physicist at Brown University, Massachusetts Institute of Technology and Brookhaven National Laboratory. Then, starting from 1972 he moved to Medicine at the Temple University School of Medicine in Philadelphia, where after a period of intensive training and research he became a tenure-track Associate Professor of Pathology (1974) and, subsequently, Full Professor and Director of the Division of Biophysics (January 1976). Alternating stages in USA (i.e., Stanford University, Nevada University), Japan (i.e., Erato Project -J.R.D.C. at Tsukuba) and Russia (i.e., Russian Academy of Sciences), he directed the Institute of Biophysics and then the Department of Biophysical M&O Sciences and Technologies of University of Genoa, after being called in 1984 to the Chair of Biophysics for "chiara fama", as "Eminent Scientist" with a special law. He was member of the National Science and Technology Council upon appointment by the Italian Parliament between 1990 and 1998. He is presently Director of the NanoWorld Institute at the University of Geneva. Over the past several years his main scientific activities concerned molecular and cellular biophysics, cancer research, bioelectronics and nanotechnology, receiving awards (e.g. American Cancer Society) and international prizes (e.g., San Valentino d'Oro); editor of several international journals, among which "Cell Biophysics" (editor-in-chief till 1991). Consultant of several International Organizations (NATO, NIH, NSF in USA, Frontiers Research in Japan, Russian Academy of Sciences, American Cancer Society); Director of twelve NATO-Advanced Study Institutes, Director from 1978 up to now of the International School of Biostructures of the Cultural Center "Ettore Majorana"; President of Industrial and International Research organizations (CIREF, Technobiochip, NWHT LLC, USA); member of national and international academies; from 1990 President of Polo Nazionale Bioelettronica - Scientific and Technological Park of Elba, from 1993 founder and President

of Fondazione EL.B.A.. From 1984 to 1987, he has been Advisor for Science and Technology of the Italian Prime Minister Craxi, and from 1988 to 1995 he was President of the National Research Program on Bioelectronics. Author of more than 641 publications in international scientific journals reviewed by the "Science Citation Index", of more than 39 WPI patents, and more than 25 books.

Conclusions

The Round Table hosted June 21, 2017 in the Fondazione ELBA Nicolini headquarter in Pradalunga (Bergamo, Italy) on USA-EUROPE Scientific Cooperation about XFEL, CRYO-EM, SYNCHROTRON RADIATION and NANOTECHNOLOGY was jointly chaired by Professor Petra Fromme from Arizona State University in USA and from Academician Claudio Nicolini from Fondazione EL.B.A. Nicolini in Europe, with the participation of Professor Christian Riekel from European Synchrotron Radiation Facility in Grenoble (France), Prof. Raimund Fromme, Arizona State University, Tempe, USA and Professor Eugenia Peshkova from Geneva University in Italy. After long scientific discussion was reached the unanimpous consensus to explore the formalization of a Bilateral Organizing Committee to meet next year in Tempe (Phoenix) at the Biodesign Institute in order to formalize a joint detailed USA-Europe research project in conjunction with the Third NanoWorld Conference planned in San Francisco for April 23-25, 2018 and to be subsequently submitted for adequate funding at the appropriate international and national agencies. This agreement concluded the ELBA NW XLII Nanoforum on Structural Nanoproteomics in the Fondazione Headquarters in Pradalunga (Bergamo, Italy) on June 22, 2017 that is being published by NanoWorld Journal in the Supplement 2 of Volume 3.

Claudio Nicolini, Editor-in-Chief NanoWorld Journal and President Fondazione ELBA Nicolini

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