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Meeting Abstracts

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Proceedings ELBA NW Nanoforum XLII on Structural NanoProteomics. Part-1: Introduction and Topics–XFEL versus Synchrotron Radiation and Cryo-EM

Introduction

The Fondazione EL.B.A. (EL.ELECTRONICS B.IOTECNOLOGY A.DVANCED) Nicolini was an initiative created by Professor Claudio Nicolini member of the National Science and Technology Council and called from USA as Eminent Scientist to the Chair of Biophysics at the University of Genova, as an outgrowth of the successful intergovernmental agreement, called "ELBA Project", on bioelectronics and biomolecular engineering signed on December 7, 1990 by the Italian Minister of Research and University Antonio Ruberti and by the Vice-Minister for Science and Technology of the Soviet Union Ivan Bortnik, and later extended to the European Commission as observer evolving into a long-range research project with multidisciplinary activities. Fondazione EL.B.A. (Electronics Biotechnology Advanced) Nicolini has been born in 1993 with participation of both governments (Europe and Soviet Union) and privates (USA Institutions) and has brought to success the initiative on Bioelectronics with the required critical mass at the world scale through (a) the support of both the multinational companies organized within Polo Nazionale Bioelettronica, Scientific Technological Park of the ELBA Island and CIREF Industrial Consortium (Sorin, Fiat, Montedison, STM, ABB, Elsag-Bailey) and (b) the Biochip Project (1) initiated by USSR President Gorbachev through Velikov-Emelyanov at Russian Academy of Sciences and Italian Prime Minister Craxi through Nicolini at Genova University and Polo Nazionale Bioelettronica. The Nanoforum is now at its XLIIth ELBA edition at Pradalunga (Bergamo) held on 21 June 2017 Aula Multimediale having as objective (www.fondazioneelba-nicolini.org) the exploration of present stage of Collaborative Lines of North American (USA in Boston and Tempe) and European (FEN NWI, LBN UNIGE and ESRF) Research, having focus in Structural NanoProteomics for Cancer and for Molecular Bioelectronics. This time the sponsorship has been extended for the first time to NanoWorld (NW) Journal and Conference both located in USA, respectively in Texas and Boston. The Joint USA-Europe Organizing Committee was chaired by Professor Petra Fromme from Arizona State University in USA and Academician Professor Claudio Nicolini from Fondazione EL.B.A. Nicolini host in Europe of this Nanoforum on Structural Nanoproteomics in the Fondazione Headquarters in Pradalunga (Bergamo, Italy) on June 21-22, 2017.

Program

10:30-12:00 Prof. Petra Fromme, Professor Arizona State University, Tempe (USA) & Desy XFEL, Germany
Keynote Lecture on XFEL and New Avenues in Protein Structure Determination

12:00-13:00 Prof. Christian Riekel, ESRF Grenoble, France
Synchrotron Radiation and New Avenues in Protein Structure Determination

13:00-13:30 Dr. Grinzato, University of Padova, Italy
Cryo-EM and Structural Biology

13:30-14:30 Lunch Break

14:30-15:30 Poster Presentations

Protein LB Crystal via NIMA vs Protective Plate	Dr. Stefano Fiordoro	University of Genova, Italy
Anodic Porous Allumina and Protein Microarrays	Dr. Claudio Larosa	University of Genova, Italy
Stability and Radiation Damage by Monte Carlo and Molecular Dynamics	Dr. Filipp Oreck	University of Genova, Italy
LB and Cancer Proteomics	Dr. Nicola Bragazzi	University of Genova, Italy

15:30-16:30 Prof. Eugenia Peshkova, Assistant Professor and Head Lab Biophysics Nanotechnology, Genova University, Italy
Protein Crystallography by Langmuir Bloghett Template

16:30-17:00 Prof. Raimund Fromme, Associate Professor, Arizona State University, Tempe, USA
Solar Light Harvesting, a New Biological Structure in Between a Reaction Center and a Photosystem

17:00-17:45 Prof. Claudio Nicolini, Fondazione ELBA Nicolini, Member Russian Academy of Sciences and President & CEO NanoWorld High Tech LLC, USA
Structural NanoProteomics by Cell Free Expression, Polymers, Enzymes, APA, Nanoconductimetry, Mass Spectrometry, LB and Montecarlo

17:45-18:15 Round Table on USA-EUROPE Scientific Cooperation on XFEL, Cryo-EM, Synchrotron Radiation and Nanotechnology
Chaired by Claudio Nicolini and extended to Petra Fromme, Christian Riekel and Eugenia Peshkova

XFEL, SYNCHROTRON RADIATION and CRYO-EM

Claudio Nicolini

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

Abstract

Time ago (in a Nature FORUM in Crystallography on January 10, 2014 Volume 505 pg.620-621) a synchrotron expert Sean McSweeney of the Department of Photon Sciences, Brookhaven National Laboratory, Upton, New York 11973-5000, USA (e-mail: smcsweeney@bnl.gov) and an advocate of free-electron lasers Petra Fromme of the Department of Chemistry and Biochemistry, Arizona State University, Tempe, Arizona 85287-1604, USA (e-mail: pfromme@asu.edu) discuss the prospects of the respective source types for applications in structural biology. Synchrotrons have long been the preferred X-ray sources for crystallography, but competition has arrived with the advent of X-ray free-electron lasers. To go deeper in the problems raised in 2014 by Nature and on the recent developments about competing XFEL, Synchrotron Radiation and Cryo-EM, I have called on June 21 and 22, 2017 an ELBA NW Nanoforum on Structural Nanoproteomics in the headquarters of Fondazione ELBA Nicolini in northern Italy with the cooperation of NanoWorld Journal who is publishing the Proceedings in its Supplement 2 of Volume 3 2017.

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Keynote Speakers

XFEL and New Avenues in Protein Structure Determination

Petra Fromme

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Abstract

Biomolecules are highly dynamic, however most structures determined so far only provide a static picture of the molecule. Serial Femtosecond Crystallography (SFX) provides a novel concept for structure determination, where X-ray diffraction "snapshots" are collected from a fully hydrated stream of nanocrystals, using femtosecond pulses from high energy X-ray free-electron lasers (XFELs). The XFEL pulses are so strong that they destroy any solid material. However, a femtosecond is extremely short (1 fs =10^{-15} s). With these ultrashort pulses X-ray damage is diminished and diffraction from the crystals is observed before destruction takes effect. The first proof of concept of serial femtosecond crystallography was achieved using Photosystem I, as a model system [1, 2]. The structure of non-damaged biomolecules can now be determined, unravelling their function at the atomic scale [3-5]. Femtosecond crystallography also opens a new avenue for determination of protein dynamics, with the goal of molecular movies of biomolecules "in action". First experiments on the proof of principle for time resolved serial femtosecond nanocrystallography have been performed on the large biosolar energy converters in Photosynthesis [6, 7] a process that converts sunlight into chemical energy and providing the oxygen and the energy for all higher life on Earth. The first snapshots of the first steps water splitting reaction have been observed by 3 research groups [7-9]. A new concept based on continuous X-ray diffraction extends resolution beyond Bragg diffraction and allows for direct phasing of X-ray diffraction data [10]. TR-SFX studies extend to atomic resolution where the first steps in photosensing were recently revealed at a time scale of femtoseconds using the photoactive yellow protein [11, 12]. This pioneering work paves the way for the determination of molecular movies of the dynamics of membrane proteins "at work" in the future including the determination of molecular movies of water splitting and also the study of reactions of enzymes [13]. The talk will close with a progress report on the development of compact femto- and attosecond X-ray sources at ASU (CXLS and CXFEL) and DESY (AXSIS) [14], which will provide unique new opportunities to study the ultrafast dynamics of reactions in photosynthesis with a combination of X-ray diffraction, X-ray spectroscopy and ultrafast optical spectroscopy.

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Biography

Dr. Petra Fromme received her B.S. (Vordiplom) and M.S. (Diplom) in Biochemistry from the Free University of Berlin, received her Ph.D. in Chemistry and did her habilitation in physical chemistry at the Technical University of Berlin. Dr. Fromme was an Assistant and Associate Professor at the Max Volmer Institute before joining Arizona State University as a full Professor in the School of Molecular Sciences in 2002. Dr. Fromme and is an affiliated member of the Department of Physics, member of the graduate faculty in the Plant Biology and Biological Design graduate programs and was awarded the Paul V Galvin Professorship in 2012 and named a Regents' Professor in 2015. Dr. Fromme was appointed by ASU President Michael Crow as the director of the Biodesign Center for Applied Structural Discovery in 2014. Dr. Fromme's research interests are focused on the study of the structure-to-function relationship of membrane proteins involved in bioenergy conversion, infectious diseases and cancer. Membrane proteins perform most of the important processes in all living cells. For example, respiration, photosynthesis, cell communication, cell import/export, cell growth and recognition are catalyzed and regulated by membrane proteins. These proteins do not act in an isolated way; they rather perform communication within the cell by binding and releasing of cofactors and soluble signal-transducing proteins. The main step for the elucidation of the complex in whole living cells is the understanding of the structure, dynamics and function of the membrane proteins that play the key role in these processes. Her research field is of a very interdisciplinary nature and includes biochemical investigations, molecular biology, spectroscopy, crystallization, X-ray structure analysis, as well as theoretical investigations. Petra Fromme's group is part of a large international collaboration, that includes the BioXFEL Science and Technology Center, who pioneers the new field of serial femtosecond nanocrystallography using Free electron lasers, where structure determination is based on femtosecond X-ray diffraction from a stream of nanocrystals, which will allow the determination of molecular movies of biomolecules at work in the future.

Protein Crystallography with Synchrotron Radiation Micro- and Nanobeams

Christian Riekel

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Abstract

The ESRF has been operating since 1994 as worldwide first 3rd generation synchrotron radiation (SR) source based on periodic magnetic insertion devices such as undulators. The ongoing machine upgrade into a quasi-4th generation SR source -called Extremely Brilliant Source (EBS)- will provide an increase of the ESRF X-ray brilliance by approximately two orders of magnitude and significantly increase the coherent beam fraction. This will in particular enhance the efficiency of micro- and nanofocusing X-ray optics and result in an important flux-increase of protein microcrystallography beamlines. I will discuss in my talk selected serial crystallography techniques which have been pioneered at X-ray Free Electron Laser (XFEL) sources and which will be extensively used at the ESRF-EBS. As compared to XFELs, SR sample environments provide considerable flexibility for probing biological objects air or even liquids. Here I will discuss selected advanced sample environments allowing positioning and probing of ultrasmall protein volumes in X-ray beams. The potential impact of digital microfluidics for probing small reaction volumes will be particularly emphasized. I will finally show that deformations introduced by the confinement of functional colloidal virus particles in a solid matrix can be probed by scanning X-ray nanodiffraction.

Biography

Christian Riekel received his Ph.D. in Chemistry at the University of Munich in 1973. He worked as research scientist for 5 years at the neutron research reactor (ILL) in Grenoble-France where he developed *in-situ* neutron diffraction techniques for studying solid-state chemical reactions. He then moved to the Polymer Department of the University of Hamburg and developed a synchrotron radiation scattering beamline at DESY for real time SAXS/WAXS studies on polymers. He joined in 1986 the founding team of the European Synchrotron (ESRF) in Grenoble and was responsible for the build-up and operation of the Microfocus beamline (ID13). Christian Riekel holds currently an emeritus scientist position at the ESRF and pursues his interdisciplinary scientific interests with an emphasis on relating microscopic structure to macroscopic function of soft and biological materials. His main interests are beta-sheet materials -like amyloids and silks- and sample environments adapted to X-ray micro- and nanobeams. Founder time ago and Director of the ID13 Beamline at the Experiments Division of the

Complex Systems and Biomedical Sciences Group at European Synchrotron Radiation Facility in Grenoble. Associate Editor of the NanoWorld Journal for Materials Soft Matter and Biological Instrumentations since 2015.

CryoEM and X-Ray Crystallography

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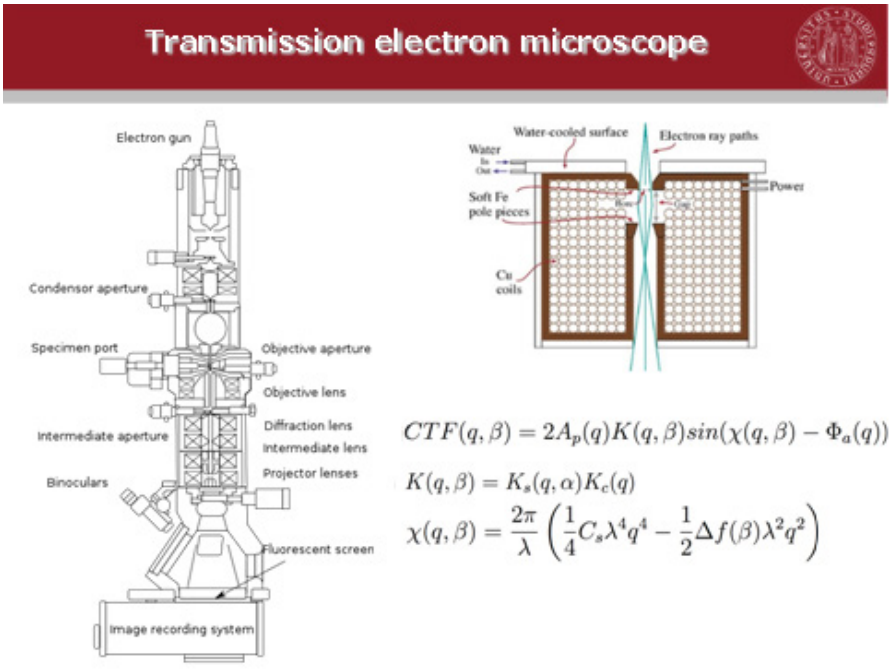
Abstract

For many years, the cryo-electron microscopy (cryoEM) was used only to determine low resolution (generally more than 10 Å) electron density maps of big proteins structure. Thanks to recent developments in electron detectors, phase plate devices, beam-induced motion correction and GPU computing [1-5], the resolution achievable with cryoEM is reaching values similar to those obtained with X-Ray crystallography (3-3.5 Å, maximum 2.5 Å) [6]. In particular, in single particles cryo-electron microscopy (SPA cryoEM) a single drop of sample randomly oriented is used to generate 2D projections of the molecule and the image processing of these projections leads to the 3D electron density map of the sample. The main advantage of SPA cryoEM is that no crystals are needed, giving the possibility to study also the structure of big and flexible macromolecules [7]. Moreover, the recent improvement in image processing [8] make possible to determine several conformational states during the same analysis. The interpretation of the 3D map obtained by CryoEM can be carried out using methodologies typical of X-Ray diffraction and already existing software [9, 10]. This is the ideal linkage between the two techniques and led to a possible new approach in proteins structure determination. In our work, we applied the Single Particles cryoEM technique to the structure of the PSII-LHCII. The PSII is a proteins complex of the thylakoid membrane involved in the photosynthesis normally active in his dimeric conformation. The PSII of plants contains a variety of peripheral complexes that include LHCII and chlorophyll-binding proteins CP29, CP26 and CP24. These proteins surround the PSII, absorb light energy and transmit it to the reaction center. The results of this study show how the SPA cryoEM can be successfully applied to difficult and inhomogeneous sample and gives an idea on how the cryoEM and X-Ray crystallography can act jointly.



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Biography

Alessandro Grinzato received his Bachelor Degree (Laurea) and Master Degree (Laurea Magistrale) in Physics at the University of Padua. His research interests are mainly focused on structural biology and molecular dynamics. During his undergraduate internship, he worked in the Professor Zanotti's Laboratory in Padua on the PSII–LHCII super complex structure reconstruction through cryoEM single particles analysis. In parallel he refined his knowledge about structural biology and applied it to several protein structures. Currently he is Research fellow at the Department of Biomedical Science of the University of Padua, where he is performing molecular dynamics studies on proteins complexes, membrane proteins and channels. Moreover, he is exploring new theoretical methods that integrates cryoEM and X-ray crystallography.

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