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Meeting Abstracts

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Applications to Space, Energy and Environment of Nano-materials, -Devices and -Systems

Nanotechnology, Bio-computing, and Space Exploration

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Abstract

The inherent need for enhanced autonomy in space systems with dramatically reduced size, weight, and power is directly benefitting from the rapid advances in microelectronics driven primarily by commercial forces in this information age. However, miniaturization of silicon devices approaching physical limits imposed by semiconductor properties suggests that the end of the Moore's law is in sight. On one hand, potential breakthroughs in molecular, DNA, and quantum computing promise phenomenal computing speed and performance, along with intriguing engineering challenges to make those a reality. On the other hand, high performance computing based on bio-inspired computing architectures is rapidly emerging as a viable, powerful paradigm for a variety of complex applications, primarily in sensory information processing. This has attracted a new generation of engineers and multi-disciplinary researchers to the study of bio-organisms. Animals and insects have their highly evolved intelligent sensory systems directly coupled to their brains: analog, highly parallel computing architectures, well-connected to their dexterous limbs and wings endowed with excellent motor control. The principle of survival-of-the-fittest has made it possible for animals and insects to evolve with phenomenal capabilities to explore and effortlessly interact with their unstructured environment. The emerging research trends in computing architectures based on the art of biomimicry are quite promising. For example, artificial neural networks with deep learning capabilities are attempting to mimic the circuitry found in animal cerebellum to provide uncanny pattern recognition, real-time control, and autonomous decision-making; solid state 3-d memories are promising unprecedented storage density and associative information recall; and evolutionary "survival-of-the-fittest" algorithms are allowing optimization of circuits and architectures, capable of reconfiguring in real-time to withstand harsh ambient conditions.

Nano-devices for Energy

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Abstract

Nano-devices can be a game-changer in energy production and harvesting when applied to large-scale commercial ground and space systems requiring reduced cost and ease in deployment. The paper presents some key concepts, outlining both benefits and challenges.

Protein Structure at Atomic Scale by Synchrotron Radiation, Cryo-EM and XFEL

X-Ray Nanodiffraction on Tobacco Mosaic Virus Assemblies

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Abstract

Protein microdiffraction (microPX) with beam sizes down to about 1 mm has become a routine technique at high brilliance synchrotron radiation (SR) sources. A further reduction of beam sizes into the nanometer range is of interest for probing ultrasmall crystallites or crystalline domains in a less ordered environment. Progress in X-ray optics, detectors and in particular the upcoming upgrade in brilliance of the ESRF suggests a considerable development potential for protein nanodiffraction (nanoPX). Here I will report on the use of nanoPX for exploring the structure and thermal degradation of plant virus nanoparticles which have been self-assembled in a confined environment. Such studies are of interest in the context of biological nanoparticles serving as building blocks for functional devices. Indeed, nanostructured electrodes with a high surface area have been assembled from tobacco mosaic virus (TMV) nanoparticles forming hollow rods of about 300 nm length and 15 nm diameter [1, 2]. We have evaporated aqueous droplets loaded with TMV capsids on wetting and superhydrophobic surfaces to generate coffee-ring type residues [3, 4]. Scanning nanoPX at the ESRF-ID13 beamline [5] with an about 200 nm beam allows probing the local structure of the residue and localize highly crystalline domains formed by capsids pinned at its outer rim. The deposition of the residue on the X-ray transparent window of a MEMS-based nanocalorimeter window [6] was used for studying *in-situ* structural changes up to about 250 °C.

References

1. Fan XZ, Pomerantseva E, Gnerlich M, Brown A, Gerasopoulos K, et al. 2013. Tobacco mosaic virus: a biological building block for micro/nano/bio systems. *J Vac Sci Technol* 31: 050815. https://doi.org/10.1116/1.4816584
2. Gerasopoulos K, Pomerantseva E, McCarthy M, Brown A, CulverJ, et al. 2012. Hierarchical three-dimensional microbattery electrodes combining bottom-up self-assembly and top-down micromachining. *ACS Nano* 6(7): 6422-6432. https://doi.org/10.1021/nn301981p
3. Gebhardt R, Teulon JM, PellequerJL, Burghammer M, Colletieret JP, et al. 2014. Virus particle assembly into crystalline domains enabled by the coffee ring effect. *Soft Matter* 10: 5458-5462. https://doi.org/10.1039/C4SM00414K
4. Marinaro G, Accardo A, De Angelis F, Dane T, Weinhausen B, et al. 2014. A superhydrophobic chip based on SU-8 photoresist pillars suspended on a silicon nitride membrane. *Lab Chip* 14: 3705-3709. https://doi.org/10.1039/c4lc00750f
5. Riekel C, Burghammer M, Dane TG, Ferrero C, Rosenthal M. 2016. Nanoscale structural features in major ampullate spider silk. *Biomacromolecules* 18(1): 231-241. https://doi.org/10.1021/acs.biomac.6b01537
6. Riekel C, Di Cola E, Reynolds M, Burghammer M, Rosenthal M, et al. 2014. Thermal transformations of self-assembled gold glyconanoparticles probed by combined nanocalorimetry and X-ray nanobeam scattering. *Langmuir* 31(1): 529-534. https://doi.org/10.1021/la504015e

Protein Structures by Cryo-EM

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Abstract

Cryo-electron microscopy (cryo-EM) has undergone an astonishing revolution in the last few years [1]. A limited, but significant gain in resolution due to the implementation of new kind of sensors that detect electrons directly, without having to convert them to photons and, in turn, in photoelectrons, has allowed electron microscopy to overcome the barrier of the 4Å - 5Å resolution [2]. A resolution close or better than 3 Å allows, in fact, to trace the polypeptide chain, which means that detailed molecular models become available. Other tools, like phase plates, and the availability of computer clusters necessary to process the huge number of images necessary for high-resolution studies, contribute to allow to reach a maximum resolution that in some cases approaches 2 Å. In cryo-EM a drop of solution of the macromolecule is frozen and molecules are observed directly on the detector. The final result of this process is the production of electron density maps that looks similar or even better than an X-ray map at the same resolution. The reason is that the phase information is contained directly in the cryo-EM images.

Among the advantages of the technique, we have to mention the fact that: i) no crystals are needed; ii) a quite limited amount of sample is enough to carry out a complete analysis; iii) large and labile macromolecular complexes can be examined and, finally, iiii) the presence of two or three different conformations of the macromolecule can be distinguished. The major drawback is, at the moment, that only large molecules (i.e., about at least 150,000-200,000 Da) can be examined and the resolution is not yet comparable with that attainable by X-ray diffraction on single crystal. Some experimental results of the process will be presented in this communication, related to large macromolecular complexes.

References

1. Stuart D, Subramaniam S, Abrescia NG. 2016. The democratization of cryo-EM. *Nat Methods* 13(8): 607-608. https://doi.org/10.1038/nmeth.3946
2. Kuhlbrandt W. 2014. The resolution revolution. *Science* 343(6178): 1443-1444. https://doi.org/10.1126/science.1251652

Protein Structure by Langmuir-Blodgett Nanotemplate

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Abstract

With the new generation of synchrotrons and microfocussed beamlines a great progress is 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. Recently, femtosecond crystallography at X-ray free electron lasers (XFELs) has opened the new way to X-ray diffraction data collection. However, the production of the protein crystals as well as their quality remain open problems. Since this field is rapidly evolving, the novel methods of macromolecule organization into the diffracting arrays (nanocrystals, 2D crystals, etc) come to the forefront.

The Langmuir-Blodgett (LB) nanotemplate method, applicable to any protein (including membrane proteins) allows highly ordered 2D LB thin protein films formation on the air-water interface and their deposition on the any solid supports, including nano-patterned chip surfaces. These templates can be applied as a 2D template for triggering of 3D protein crystals, including direct "on chip" protein crystals preparation. The specific properties of LB protein thin films (long range order, thermal stability, ability of trigger protein crystallization including those non-crystalizable by classical methods) can be exploited in new procedures for fix target serial femtosecond crystallography. Thereby, LB protein nanotemplate approach includes the diffraction data collection from nanocrystal grown by LB nanotemplate, single and multilayer LB film, deposited onto the nano-patterned ad hoc designed chips, aiming the protein structural data collection at crystallographic resolution. 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 give new insights to the of LB nanotemplate phenomena and its ability to trigger and accelerate protein crystallization.

The combination of LB technology with such advanced technologies as XFEL and Cryo-electron microscopy with direct detectors has the potential to become the important tool for the structure determination of proteins that are difficult to crystallize, such as membrane proteins.

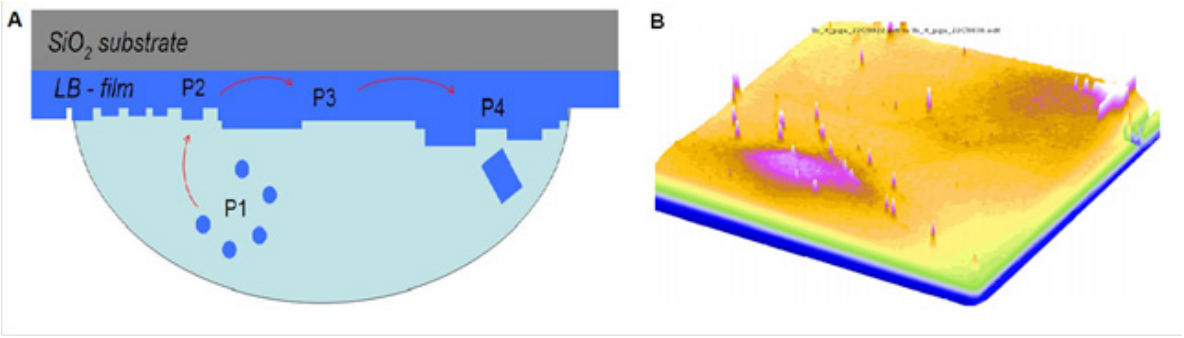

Figure 1: (A) The temporal model of LB nanotemplate based on in situ submicroGISAX measurements, where the protein solution, P1, leads to protein association on the LB film states, P2 and P3, and to the crystal formation P4 detaching from the film in the drop. **(B)** 3D representation of the area detector data for protein LB multilayer with long-range order showing clear diffraction peaks.

References

1. Pechkova E, Riekel C. 2012. Synchrotron Radiation and Structural Proteomics. Pan Stanford Series on Nanobiotechnology, Volume 3, Singapore.
2. Pechkova E, Zanotti G, Nicolini C. 2003. Three-dimensional atomic structure of a catalytic subunit mutant of human protein kinase CK2. *Acta Crystallogr* 59(Pt 12): 2133-2139. https://doi.org/10.1107/S0907444903018900
3. Pechkova E, Nicolini C, 2004. Protein nanocrystallography: a new approach to structural proteomics. *Trends Biotechnol* 22(3): 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(Pt 3): 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(3): 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. *Biophys J* 99(4): 1256-1261. https://doi.org/10.1016/j.bpj.2010.03.069
7. 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
8. Pechkova E, Nicolini C. 2017. Langmuir-Blodgett nanotemplates for protein crystallography. *Nat Protoc.* (Accepted).

Applications of Synchrotron Light

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Abstract

Synchrotron Sources worldwide provide an ideal platform to study and characterize biological systems. An overview of the methods available at synchrotron radiation facilities to study life sciences will be presented. We will discuss the complementarity between those synchrotron methods and those found at individual Laboratories or Large Centers, such as microscopy or spectroscopic techniques.

Unfolding the Nanoworld of Stem Cells Towards a Self-Healing Potential

Unfolding the Nanoworld of Stem Cells: Electromagnetic and Acoustic Communication for a New Paradigm in Regenerative Medicine

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Abstract

Cell polarity in somatic and stem cells results from and acts on the modulation of cellular ion fluxes, electric fields, and nanomechanics of the cytoskeleton and nucleoskeleton. Cell polarity is also crucial in epigenetic modulation of stem cell differentiation and aging. Here, we will discuss our recent findings showing that properly conveyed radioelectric fields are able to: (i) enhance the differentiating potential of mouse embryonic stem cells, (ii) induce pluripotency in human adult stem cells, promoting their differentiation into cardiac, neural, skeletal muscle and endothelial cells, (iii) afford direct reprogramming towards the same lineages in human somatic cells (dermal fibroblasts), (iv) reverse human stem cell aging in vitro, (v) reprogram PC12 cancer cells into dopaminergic neurons, and (vi) optimize stem cell polarity.

We will also discuss the role of mechanical vibrations in the modulation of (stem) cell signaling networks and cellular expression of multilineage potential. On the whole, we would like to present an evolving picture of cells capable to perceive themselves as a rhythmic component of the universe, sensing and producing magnetic fields and sound vibrations, progressing through transition states interspaced by the emergence of ordered images of structure and function. We can now govern the appearance of these images with electromagnetic and acoustic energies. Due to their diffusive nature, we are able to target and reprogram the stem cells where they are, in all tissues of the body. This strategy promotes our natural ability for self-healing, affording a regenerative medicine without the needs for stem cell transplantation.

Nanomaterials with Tunable Hydrophobicity as Local Drug Delivery Systems for Cell Transplantation

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Abstract

Self-assembly represents one of the most important driving forces in biology, regulating processes like formation of cellular

membranes. Inspired by nature's self-assembly, we developed an innovative nanomedicine platform of polymeric self-assembling nanomaterials for novel applications in cell transplantation for type-1 diabetes. Self-assembling block-copolymers are macro-amphiphiles able to form supra-molecular structures when dispersed in water. The morphology of block-copolymers can be easily varied to modulate their capacity to encapsulate and release small molecules, polypeptides, and nucleic acids for local drug delivery. In addition, the chemistry of block-copolymers can be modified in order to control their stability and to reduce their toxicity. In type 1 diabetes (T1D) replacement of pancreatic islets through transplantation requires life-long immunosuppression and graft survival is limited. Local immunomodulation by targeted delivery of immunomodulatory molecules to lymph nodes draining the islet graft site and/or in the graft site may reduce or even eliminate the need for systemic and chronic immunosuppression. Here, we show the fabrication of self-assembling polymeric biomaterials made of polyethylene glycol and polypropylene sulfide (PEG-PPS) or PEG and olygoethylene sulfide (PEG-OES) designed for efficient encapsulation and for local controlled delivery of immunomodulatory drugs in the peri-transplant space. We demonstrated the ability of this drug delivery system to traffic to lymph node-resident immune cells and target specific immune cell populations prolonging a skin-allograft survival, as well as their ability to efficiently transfect DNA in vitro and in vivo in a tumor immunotoxicity model in mice.

One Step Tissue Engineering with Combined ADSC and BMSC in Severe Osteoarthritis for Cartilage Repair

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Abstract

An innovative personal clinical experience has provided evidence that widest areas of cartilage reconstruction in severe defects due to osteoarthritis of the inferior limb, Kellgren stage I to III, in particular knee and ankle, can be successfully restored through the combinatorial transplant of human bone marrow and the Lipogems product: the last is a microfractured autologous human fat containing an intact stromal vascular niche, including elements of pericyte identity (Lipogems EU, http://lipogems.eu). Both are embedded within nanofabricated scaffolds with tailored oriented architecture and fiber diameter (Chondrotissue by Biotissue, Freiburg D, http.//www.biotissue.de). Such approach yields a significantly more enhanced cartilage regeneration, as compared with the rescuing effects elicited by either bone marrow or the Lipogems product alone. All these clinical outcomes are well documented by 1.5 NMR, elastosonography and in some randomized histological samples: the last documenting the highest percentage of hyaline cartilage and rare fibrous tissue in confront of the outcome of the single, not combined procedures. On these bases, the combinatorial use of autologous non-expanded tissue products made of whole bone marrow and human adipose tissue derivatives, such as the microfractured Lipogems product, can be considered as an autologous/homologous strategy for improving the natural capacity for self-healing in damaged osteo-articular tissues.

Living on Plastic: The Unnatural History of Mesenchymal Stem Cells

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Abstract

Mesenchymal stem cells (MSCs) appear after a few weeks of culture of any enzyme-dissociated vascularized tissue. The method is simple and efficient, but has left the identity of the native MSC unknown. We and others have shown that innate presumptive MSCs live in a perivascular "niche". We have identified both pericytes (PCs), which encircle microvessels, and adventitial stromal cells (ASCs), located in the outmost layer of arteries and veins, as the origin of MSCs. Purified PCs and ASCs are indeed indistinguishable, after culture, from conventional MSCs. Transcriptome analysis on single perivascular cells sorted from adipose tissue has shown that PCs and ASCs are arranged as a hierarchy in which adventitial cells are the most primitive. Clonal culture and RNA sequencing have also revealed that in vitro switch of PCs and ASCs into MSCs is accompanied by dramatic changes including clonal selection and strong modifications in gene expression. Altogether, these results reveal that MSCs are profoundly affected by in vitro culture. Several studies have already documented the advantage of using sorted PCs and ASCs in place of conventional cultured MSCs for tissue repair and regeneration. Finally, we have used supramolecular hydrogels to induce the differentiation of purified human pericytes. Changing hydrogel stiffness from soft to hard resulted in differentiation into either neural, chondrogenic or osteogenic cell lineages. We investigated the metabolome of these differentiation cultures, and identified bioactive metabolites that can specifically target bone and cartilage formation.

Regulation of the Adaptive Response of Cardiac Cells to Ischemia: Role of Nanovesicles

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Abstract

Ischemia-induced myocardial injury contributes to perioperative morbidity and mortality during organ transplantation, cardiac and general surgery. An imbalance in cellular cross-talk within the ischemic myocardium results in severe tissue injury and contractile dysfunction, which are further enhanced during subsequent reperfusion. Even if the adult myocardium is formed by different type of cells, the regulation of local paracrine activity confers a protection of the cardiomyocytes against a subsequent lethal injury. We have recently demonstrated that exosomes, nanovesicles enclosed by a plasmalemma-derived membrane, are paracrine regulators of ischemic tolerance in the heart. Exosomes interact with several molecular pathways and deliver key mediators of cell-cell communication like a biologically active Trojan horse. The exosomal cargo is composed of proteins (transcriptional factors, enzymes, heat shock proteins) or, oligonucleotides (DNA, mRNA, microRNAs and noncoding RNA) or metabolites (lipids, lactic acid and glutamic acid). In addition, exosomes transfer plasma-membrane ligands or activated G-protein coupled receptors, which may activate pro-survival signaling pathways in a dose and time-dependent manner. Exosomes released from cells with different phenotype exert different function in order to preserve cardiac function. In particular, we have observed that cardiac progenitor cells are more able to release pro-survival exosomes compared to fibroblasts and cardiomyocytes. Conversely, cardiac fibroblasts are more able to mediate the hypertrophic response of cardiomyocytes. In conclusion, the cardiac exosomes show great promise towards nanomedicine for patient-specific cardioprotection.

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