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Meeting Abstracts

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Exploration of the Nano-world of Biomolecules "in Action" with Free Electron Lasers

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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). To illustrate this time domain just consider that the time difference between a fs and a second is the same as between a second and 32 million years. With these ultrashort pulses X-ray damage is "outrun" and structural information (i.e. diffraction) from the crystals is observed before destruction takes effect [1]. The first proof of concept of serial femtosecond crystallography was achieved using Photosystem I, a larger membrane protein complex involved in Photosynthesis as a model system [2, 3]. The structure of non-damaged biomolecules can now be determined, unravelling their function at the atomic scale [4, 5] that include important human membrane-bound receptors that are key players in vision (rhodopsin-arrestin) [6], hormone function (serotonin receptor) [7], control of blood pressure (Angiotensin receptor) [8], development of cancer (smoothened receptor) [9] as well as receptors that may provide new avenues against drug abuse (opioid receptor) [10].

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, a process that converts sunlight into chemical energy and providing the oxygen and the energy for all higher life on Earth [11, 12]. The first snapshots of the first steps water splitting reaction have been observed [12, 13]. TR-SFX studies extend to atomic resolution where the first steps in photosensing were recently revealed at a time scale of femtoseconds sing the photoactive yellow protein [14, 15]. 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. The talk will close with a report on the development of compact femto and attosecond X-ray Sources, which will provide unique new opportunities to study the ultrafast dynamics in biology with a combination of X-ray diffraction, X-ray spectroscopy and ultrafast optical spectroscopy.

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Fundamental and Industrial Nano approaches to Cancer from Nanolab to Patient

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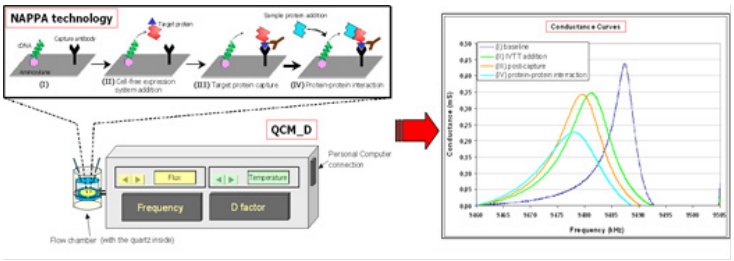
Abstract

Cancer is still facing tremendous challenges at fundamental and industrial level despite the huge body of research and allocated resources, with 14.9 million incident cancer cases, 8.2 million deaths, and 196.3 million disability-adjusted life years in 2013. The discovery of biomarkers and molecular signatures originating from Nanoproteomics and Nanogenomics is leading to new, individualized avenues from the advanced nanolab to single patients [1].



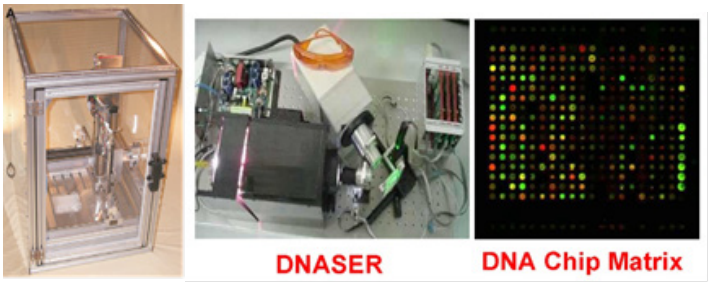
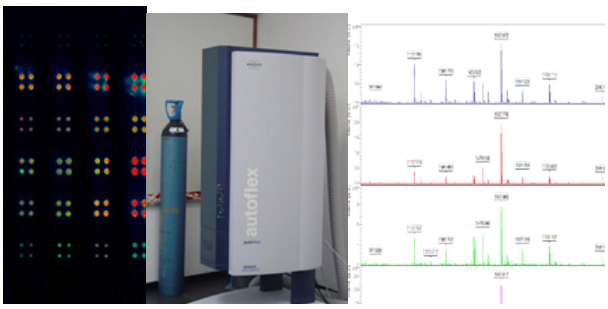


Figure 1: (Above) Flow-cell and static dual QMC_D prototype built in house to follow each step of the protein expression versus time; (below) Identification of leading genes by bioinformatics and DNASER prototype for fluorescence analysis (left, above) of genes microarray (right, above). Interaction network for genes distinguishing lymphoma from normal T cells. Subnetwork connecting the four leader genes which are "neutral" according to their expression pattern are shown with a dotted line.

The first novel technology we introduced, namely quartz-crystal microbalance with dissipation factor monitoring (QCM_D), is a conductometric device successfully coupled with cell-free *in vitro* transcription and translation (IVTT) systems [2-4], such as Nucleic Acid Programmable Protein Array (NAPPA). The powerful instrument shown in Figure 1 can be effectively exploited for implementing biosensors and discovering new potentially clinically relevant biomarkers, being able to perform both genomics and proteomics tasks and to distinguish among different proteins or other compounds, each one having its unique conductance curve. With QCM_D we studied, among others, Atf2 (Activating transcription factor 2), p53 (tumor protein 53 kDa), MLH1 (MutL homolog 1, colon cancer, nonpolyposis type 2), PolD (Family D DNA polymerase), and Jun [5], which are frequently mutated in colorectal cancer, another focus of our research. Atf2 encodes an enzyme catalyzing acetylation and is involved in chromatin remodeling and epigenetic modifications induced by some carcinogenetic compounds, leading to oral cancer. p53 is known as the "guardian of the genome" and its mutation leads to several malignancies, including colorectal cancer. The second novel technology we introduced, namely Mass Spectrometry [6, 7], presents a useful approach towards for biomarkers identification in a self-assembling protein microarray based on NAPPA and SNAP tag coupled to E.coli cell free expression system , proving capable to resolve the "background" problem associated to the above label free detection system for the identification of proteins and of protein-protein interaction in humans that appears useful in clinical practice (Figure 2) with integrated ad hoc software Spectrum Analyzer and Data Set manager (SpADS), enabling to identify protein being expressed by fingerprinting after subtraction of the recombinant bacterial lysate present in the recombinant Pure Express.



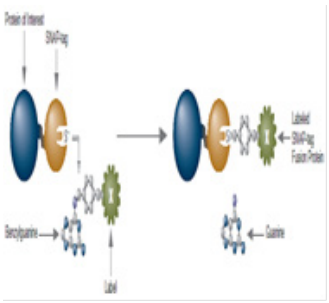


Figure 2: Mass Spectrometry (left) Samples were printed on a gold coated glass slides; the array printing was realized in a special geometry for MS analysis. The spots of 300 microns were printed in 12 boxes of 10x10 printed with SNAP genes (p53, CDK2, Src-SH2 and PTPN11-SH2), lower boxes were printed with master mix as negative control. (center) SNAP-NAPPAs were analyzed by MALDI-TOF MS. For Bruker MS analysis the matrix was mixed with the trypsin digested fragment solutions directly on the slides and let to dry before the analysis. (right) SNAP-tag is a tool for the specific, covalent attachment of virtually any molecule to a protein of interest. A single gene construct yields a tagged fusion protein capable of forming a linkage to functional groups.

Piezoelectric inkjet printing offers the possibility of spotting on complex surfaces and using clinical samples of limited volume and amount, while QCM_D discriminates several genes per spot contrary to the single one with fluorescence. We investigated well known pairs of interacting molecules like Cholesterol side-chain cleavage enzyme 11A1 (CYP11A1) and Cholesterol, Jun and Activating Transcription Factor 2 (ATF2), p53 and MDM2. An interesting implication for potential clinical applications concerned furthermore the possibility to drastically reduce the time of protein expression and capture under our experimental conditions.

Protein-protein interactions play indeed a major role in Cancer Control and their detailed understanding by Label-Free Nanotechnology is essential especially within the framework of a personalized medicine-based approach [7] using the New

England BioLabs SNAP and Pure Express-based Genes Expression in conjunction with "sub-micron arrays" based on Anodic Porous Allumina [8, 9] to exploit our industrial Label Free Nanotechnologies (Figure 3).

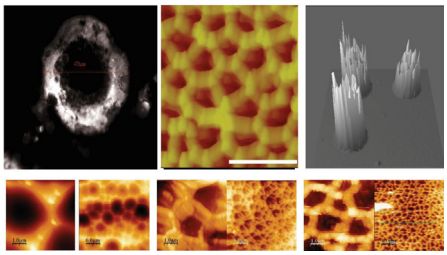


Figure 3: Single NAPPA fluorescence genes printed in microarray APA (left) after its expression in 2D (center) and 3D (right), with the different pore size and depth shown below for an optimal industrial approach.

At the same time Autoflex Mass Spectrometry shows discrimination of NAPPA versus SNAP arrays and of different genes as p53 versus CdK2 genes expressed using PURE E. Coli system. Due to the high complexity of the system, very difficult results were obtained and, therefore, in order to reduce this complexity and make sense of the findings, there was the need to develop and design an ad hoc bioinformatics tool for the analysis. The two combined Label Free technologies, Mass Spectrometry integrated by proprietary software and technology, as new ad hoc proprietary bioinformatics tools called SpADS , and QCM_D Nanoconductometry integrated by proprietary hardware and software, as applied to optimized microarray with micron sized genes spots using SNAP and bacterial lysate technologies further optimized in this application to industrial program, may be constructed in such further optimized way using single gene, oversized spots to overcome multiple gene expression and interaction in a fashion unmatchable by current fluorescence technology.

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