SOLEIL Users' Meeting, Satellite Workshop 2015

INRA-SOLEIL: The Synchrotron Approach in Agriculture, Food and Environment Sciences

(INRA-SOLEIL2015)

January 21st, 2015
Synchrotron SOLEIL, Saint-Aubin – France

Topics

- Environment and sustainable development
- New opportunities for life science at SOLEIL
- Biology Bioresources
- Nutrition, health

Local Organizing Committee

BULEON Alain, CHARDOT Thierry, DUMAS Paul, FRAISSARD Frédérique, GIULIANI Alexandre, LEGRAND Pauline, LUCACCHIONI Jean-Marc, METERIAN Iskouhie, ROBLIN Pierre, YAO Stéphanie

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www.synchrotron-soleil.fr/Soleil/ToutesActualites/Workshops/2015/INRA-SOLEIL2015/Welcome

Welcome

INRA-SOLEIL: The Synchrotron approach in agriculture, food and environment sciences

INRA and SOLEIL synchrotron facility have undertook and maintained a fruitful partnership since 2006. A one- day meeting entitled « INRA-SOLEIL: the synchrotron approach in agriculture, food and environment sciences » will be held on Tuesday 21st of January 2015, as a satellite of the 10th SOLEIL User's Meeting.

The SOLEIL synchrotron radiation beamlines and especially those more dedicated to biology provide a unique experimental environment allowing to combine complementary experiment approaches. Synchrotron radiation offers the possibility to probe matter from the atomic ,and molecular scale up to the cellular and tissue level. Societal questions such as sustainable development, food, environment, and animal health are addressed through these researches.

The meeting is opened to the wide scientific community, fees-free and aims at presenting the ongoing status of these themes , in relation with the beamlines involved.

The INRA-SOLEIL meeting will be opened jointly by the SOLEIL CEO Jean Daillant and by the INRA Deputy CEO Olivier Le Gall.

The day will be structured around a dozen of plenary invited conferences devoted to the main themes identified and will aim at illustrating the complementarity of the existing and future beamlines.. A session will be dedicated to novel opportunities at SOLEIL for life science.

Bienvenue

INRA-SOLEIL : L'approche Synchrotron en agriculture, alimentation et environnement

L'INRA et le synchrotron SOLEIL entretiennent un partenariat fructueux depuis 2006. A l'occasion du 10e Colloque des Utilisateurs de SOLEIL, une journée de colloque satellite intitulé «INRA-SOLEIL» l'Approche Synchrotron en Agriculture, Alimentation et Environnement» est organisée le mercredi 21 janvier 2015 à l'Amphithéâtre du synchrotron SOLEIL.

Les différentes lignes de lumière de SOLEIL et notamment celles dédiées à la biologie proposent un environnement expérimental unique permettant d'associer plusieurs approches complémentaires. Le rayonnement synchrotron offre la possibilité de sonder la matière de l'échelle atomique et moléculaire jusqu'au niveau cellulaire et tissulaire. À travers ces recherches sont abordées des questions de sociétés comme le développement durable, l'alimentation, l'environnement ou la santé animale.

Cette journée est ouverte à l'ensemble des communautés, sans frais d'inscription, permettra de faire le point sur ces grandes thématiques et sur les lignes de SOLEIL impliquées.

La journée sera ouverte conjointement par Jean Daillant, Directeur Général de SOLEIL et par Olivier Le Gall, Directeur Général Délégué de l'INRA.

Elle s'articulera autour une douzaine de conférences orales sur invitation traitant des grandes thématiques et illustrant la complémentarité des lignes de lumière. Une session sera dédiée à la présentation des nouvelles opportunités pour les sciences du vivant à SOLEIL.

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Summary

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SOLEIL Users' Meeting, Satellite Workshop 2015

INRA-SOLEIL: The Synchrotron approach in agriculture, food and environment sciences (INRA-SOLEIL 2015)

January 21st, 2015

Synchrotron SOLEIL, Saint-Aubin - France

Programme

08:45 - 09:25	Registration & coffee
09:25 - 09:30	Welcome / Introduction **Andrew Thompson** – SOLEIL Life Science Scientific Director
09:30 - 09:45	The word of INRA General Deputy Director for Scientific Affairs **Olivier Le Gall**
09:45 - 10:00	The word of SOLEIL General Director **Jean Daillant**

Session I : Environment and Sustainable Development

Chairperson: Thierry Chardot

10:00 - 10:20	A trace elements-based approach to tropical dendrochronology ***Stéphane Ponton*** *- (INRA, Champenoux, France)*
10:20 - 10:40	Deciphering iron loading in seeds and discovery of a new pool of iron in the nucleolus with Synchrotron Radiation X-ray fluorescence ***Stéphane Mari*** *- (BPMB, Montpellier, France)*
10:40 - 11:10	*Coffee break*
11:10 - 11:30	Yeasts: Mini-factories producing tailored lipids for green chemistry. When infrared light reveals cell metabolism ***Marine Froissard*** *– (INRA, AgroParisTech, Versailles, France)*
11:30 - 11:50	Macromolecular orientation in shape memory starch materials by infrared microspectroscopy ***Denis Lourdin*** *- (INRA, BIA, Nantes, France)*

Session II: New Opportunities for Life Science at SOLEIL

Chairperson: Paul Dumas

11:50 - 12:10	Fast scanning multi-technique spectromicroscopy possibilities at the NANOSCOPIUM beamline of Synchrotron SOLEIL ***Andrea Somogyi*** *- (Synchrotron SOLEIL, Gif-sur-Yvette, France)*
12:10 - 12:30	The ANATOMIX beamline project at SOLEIL. Phase-contrast tomography and full-field X-ray microscopy ***Timm Weitkamp*** *- (Synchrotron SOLEIL, Gif-sur-Yvette, France)*
12:30 - 14:00	*Lunch*

Session III: Biology, Bioresources

Chairperson: Monique Axelos

14:00 - 14:20	Time resolved high resolution 3D imaging of enzymes working in situ ***Alain Buléon*** *- (INRA-BIA, Nantes, France)*
14:20 - 14:40	Molecular analysis of the bacillus subtilis minimal NHEJ repair complex ***François Lecointe*** *– (MICALIS, Jouy-en-Josas, France)*
14:40 - 15:00	Structural studies of proteins inserted in a half membrane using SOLEIL Synchrotron light ***Thierry Chardot***,*- (INRA AgroParisTech, Versailles, France)*
15:00 - 15:20	PB1-F2 influenza a virus protein beta aggregated secondary structure: Synchrotron infrared and fluorescent microscopy in infected-cells ***Christophe Chevalier*** *- (INRA-VIM, Jouy-en-Josas, France)*
15:20 - 15:40	*Coffee break*

Session IV: Nutrition, Health

Chairperson: Alain Buléon

15:40 - 16:00	Towards a better understanding of the cryo-resistance of lactic acid bacteria by using infrared and UV Synchrotron Radiation ***Fernanda Fonseca*** *– (INRA AgroParisTech, Thiverval-Grignon, France)*
16:00 - 16:20	High-resolution imaging of mechanical properties using a mid-infrared Synchrotron Radiation ***Olivier Vitrac*** *– (INRA, Massy, France)*

16:20 - 16:40	Astringency and the interactions between a human salivary proline-rich protein and tannins ***Francis Canon*** *- (INRA, CSGA, Dijon, France)*
16:40 - 17:00	Insights into the organization of the respiratory syncytial virus polymerase complex: Structural characterization of the N/RNA-P and N^0-P complexes ***Marie Galloux*** *- (INRA-VIM, Jouy-en-Josas, France)*
	Round Table
17:00 - 18:00	**Monique Axelos**, INRA, Head of division **Andrew Thompson**, SOLEIL Life Science Scientific Director **Gilles Aumont** , INRA, Director of research infrastructures **Matthieu Réfrégiers,** SOLEIL, DISCO Beamline Scientist
18:00 - 18:05	**Conclusions**

ABSTRACTS

A Trace Elements-based Approach to Tropical Dendrochronology

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ABSTRACT

Because of their dominant role in the global carbon cycle, changes in tropical forest dynamics can affect the pace of the climate changes[1]. Today, the only estimation of tropical tree growth comes from permanent plot networks. However, their installation is still much too recent (i.e. beginning of the 80's for the oldest stations) to provide long enough temporal series to answer primordial, yet unsolved questions. Is tropical tree growth controlled by climate variations? Are tropical forests showing long-term changes of their productivity as temperate forests do? What are the impacts of the global changes on tropical forest functioning? An even more basic question is still fully unsolved: how old are tropical trees? Nevertheless, not any sustainable exploitation and management of the tropical forest resources is conceivable without knowing the age of the harvested trees.

The dendrochronology approach (i.e. the study of annual rings in trees) was remarkably successful in solving these questions for temperate climate trees. The retrospective analysis of tree growth patterns not only gives direct access to the age of the trees, but allows reconstructing the past effects of the environment on tree physiology over decades or even centuries. However, insurmountable difficulties to identify annual growth rings in most tropical trees (due to nearly constant growth rates over the year) prevented dendrochronologists from using the classical method of ring width measurements[2]. As we urgently need to understand the effects of the ongoing changes in climate and atmosphere on tropical forest, it appears especially important to develop new techniques for tropical dendrochronology.

The objective of the project was to evidence the existence of cyclic chemical signals in tropical wood that could compensate for the lack of visible growth rings. The potential of dendrochemical analysis for emphasizing reliable markers of seasonal cycles was tested on trees of known age from three different species: two major long-lived species of the Guianan-Amazon tropical wet forest (*Dicorynia guianensis*, *Sextonia rubra*) and one from the equatorial African forest (*Pericopsis elata*). The analyses were performed at the DiffAbs beamline using synchrotron monochromatic X-ray beam of energy 18.1 keV. For each wood sample, the radial distribution of 17 elements was analyzed from the bark to the pith with a 300 µm spatial resolution using a Silicon Drift Detector.

The results showed that these ringless tropical trees, by displaying seasonal variations in wood element composition, can yet keep an archive of their past growth. Compared to the only previous report[3] established on one single tree, the current study, based on 13 trees, constitutes a much more robust assessment.

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Deciphering Iron Loading in Seeds and Discovery of a New Pool of Iron in the Nucleolus with Synchrotron Radiation X-ray Fluorescence

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ABSTRACT

IRON SHINES UNDER LUCIA'S LIGHT

Iron (Fe) is an essential element for all living organisms, being the main cofactor of electron transfer proteins of photosynthesis, respiration and biosynthetic pathways. The control of Fe distribution to the different subcellular compartments (chloroplasts, mitochondria…) is crucial to maintain the metabolic activity of plant cells, particularly in conditions of Fe deficiency. One main challenge is thus to decipher how Fe is delivered to each compartment, where it is localized and what is its chemical environment. In this context, there is a specific need for analytical approaches to unravel two key aspects of Fe homeostasis: high-resolution imaging and *in situ* speciation.

We have used seeds from pea (*Pisum sativum*) as a model to study the process of Fe transport and localization, since these seeds are particularly Fe-rich and their size is compatible with the constraints of synchrotron beam lines. Thanks to the access to the LUCIA beamline, we have been able to characterize the chemical forms of Fe that are delivered to the embryo during the seed filling process and we have obtained crucial information on the subcellular localization of Fe. We have shown by EXAFS (Extended X-ray Absorption Fine Structure, LUCIA) and mass spectrometry that Fe is delivered to the developing embryo as ferric (FeIII) complexes with citrate and malate (1). This information was pivotal to further dissect how Fe is imported to the embryo. Indeed, we have unravelled the central role of ascorbic acid in the reduction of the Fe(III) complexes for the transport of Fe(II) by embryos. In the cells, we have discovered with µXRF imaging the existence of an unexpected pool of Fe located in a subcellular structure that was proven to correspond to the nucleolus, raising the question of the role of Fe in this specific compartment (2, 3).

In conclusion, the results obtained with the LUCIA beam line have led us to important discoveries in plant biology, paving the way for new mechanisms and roles of iron in plants.

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Yeasts: Mini-factories Producing Tailored Lipids for Green Chemistry. When Infrared Light Reveals Cell Metabolism.

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ABSTRACT

Yeasts have been used for centuries in biotechnological applications, for food processing industry (wine, beer, cider, bread, cheese, etc.). For the past few decades, they have also been studied with the aim of producing oil to replace fossil resources.

Through a genetic modification of baking yeast (*S. cerevisiae*), i.e. expression of proteins involved in oil storage in seeds, we obtained strains able to produce more lipids [1]. To better understand the mechanisms involved in oil accumulation in these yeasts, we analysed these strains and control strains (with low oil content) by infrared microscopy (Fourier Transformed Infrared, FT-IR). This technique provides an overview of the cellular metabolism thanks to a spectral fingerprint of biological macromolecules (lipids, nucleic acids, sugars, etc.). FT-IR can replace traditional biochemical tests, which use large quantities of biomass and solvents. Large populations can be tested (high throughput approaches). Combined with the high spectral and spatial resolution offered by synchrotron light, FT-IR can be used for an analysis at the single cell scale, for statistical and population heterogeneity analyses.

Thanks to the presence of the biology lab in the SOLEIL facility, we were able to develop a protocol allowing the FT-IR analysis of freshly-sampled cells at different growth times. A drop of the cell suspension was dried on a ZnSe ATR hemisphere 4 mm in diameter. The spectra were recorded using the Continuum XL microscope available on the SMIS beamline, with a spatial resolution of 4x4 µm, which is the mean size of a cell. The spectra were then analysed (spectrum correction and statistical analyses) to evaluate spectrum variations between strains. We have shown that an increase in oil content leads to significant metabolic changes, in particular on carbon pools. An inverse correlation between oil content and carbohydrates reserves (glycogen) was revealed. This inverse correlation between storage lipids and storage carbohydrates has been confirmed by biochemical analysis [2].

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Macromolecular Orientation in Shape Memory Starch Materials by Infrared Microspectroscopy

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ABSTRACT

In previous work we demonstrated the remarkable shape memory properties of starch materials, stimulated by temperature or humidity[1]. Shape memory corresponds to the ability for a material to recover a "programmed" permanent shape from a temporary one, when submitted to an external stimulus[2]. This effect is usually attributed to the presence of molecular rigid domains and flexible domains which can be oriented and freeze, respectively above and below the glass transition temperature. The objective of this study was to elucidate molecular mechanism in order to optimize raw material and its transformation.

Macromolecular conformation of extruded starch samples in permanent and temporary shape was characterized by synchrotron radiation polarized infra red microspectroscopy. Infra red spectra in the reflexion mode was recorded in parallel and perpendicular direction of the incident beam. PCA statistical analysis realized on a wide number of experiments demonstrated significant differences between permanent and temporary shape. Results confirm the presence of molecular orientation in temporary shape as detected by WAXS but at a much higher resolution (12 µm). It shows that amorphous domains are mainly involved in the shape memory effect but also that some ordered domains are present within the samples. The presence of infrared sharp bands linked to hydrogen bonds and α(1-4) linkage evidence the presence of local order which could be under the form of helical fragments potentially stabilized by intramolecular H bonds[3].

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Fast Scanning Multi-technique Spectromicroscopy Possibilities at the NANOSCOPIUM Beamline of Synchrotron SOLEIL

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ABSTRACT

The Nanoscopium 155 m long beamline of Synchrotron Soleil is dedicated to scanning hard X-ray nanoprobe techniques. Nanoscopium aims to reach 30-200 nm resolution in the 5-20 keV energy range for routine user experiments. Multi-technique scanning imaging and tomography including X-ray fluorescence spectrometry, absorption, differential phase contrast and dark field are implemented at the beamline in order to provide simultaneous information on the elemental distribution, speciation and sample morphology. The proof of principle multi-technique fast scanning imaging and tomography experiments performed with ms dwell time per pixel and the possibilities of multimodal imaging at Nanoscopium will be presented in this talk.

The ANATOMIX Beamline Project at SOLEIL – Phase-contrast Tomography and Full-field X-ray Microscopy

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ABSTRACT

Synchrotron SOLEIL is currently building a new long beamline named ANATOMIX, dedicated to coherent X-ray tomography in absorption and phase contrast and to hard X-ray microscopy. It will operate in an X-ray energy range from 5 to 25 keV, suitable for 3D imaging of heterogeneous materials at the micro- and nanoscale, and of organic matter up to several cm in thickness.

A sophisticated beam transport concept ensures optimized flux density and coherence conditions for all accessible length scales, an essential prerequisite for the investigation of hierarchical structures, as well as for time-resolved fast tomography.

The beamline will take X rays from an undulator in a canted straight section of the Soleil storage ring shared with the nanofocus beamline "Nanoscopium". The experimental facilities will be housed in a common satellite building.

Two experimental end stations will provide several complementary tomography methods. One of the stations will be optimized for X-ray microscopy down to a pixel size of 30 nm; the other, at more moderate spatial resolution, will provide parallel-beam microtomography with X-ray beams of different sizes up to a maximum beam width of 40 mm. High contrast will be obtained through inline phase contrast and X-ray grating interferometry (XGI).

By bridging the gap between well-established, but invasive high resolution methods such as electron microscopy and less resolving 3D methods such as laboratory X-ray microtomography, the ANATOMIX beamline meets the needs of users from materials science and biomedical research, as well as many other fields.

The construction of ANATOMIX is largely supported by the Agence Nationale de la Recherche (ANR) of the French State through the EQUIPEX investment program, project "NanoimagesX", grant no. ANR-11-EQPX-0031. The "NanoimagesX" consortium has 17 member partners from public research institutions and industrial companies.

Time Resolved High Resolution 3D Imaging of Enzymes Working In Situ

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ABSTRACT

Starch is the major energy reserve of a large variety of higher plants. It is the predominant carbohydrate in our food. It is also being increasingly used in many non-food issues like pulp and paper industry, pharmaceutical applications, adhesives, bio-based materials, biofuels etc. Starch has a complex semi-crystalline granular structure exhibiting size and shape dependence on botanical origin.

Enzymatic hydrolysis of native starch is involved in many biological and industrial processes as for example starch metabolism in plants, digestion by mammals, malting, fermentation, glucose syrup or bioethanol production. The hydrolysis of native starch strongly depends on starch structure and amylase source. The morphology and the surface of the granule, the amylose content, the crystalline structure or the presence of amylose lipid complexes were shown to be limiting factors to hydrolysis of the starch granule. Two new alpha-amylases from *Rhizomucor species* (RA) and *Anoxybacillus flavothermus* (AFA), found to be effective in biofuel and low temperature glucose syrup production, were studied in detail due to their very high efficiency on concentrated suspensions of native raw starch granules below gelatinization temperature. Hydrolysis rate reaches 85% for 31% maize starch suspensions.[1,2]

The location of the enzymes at different hydrolysis times within single starch granules was determined using tryptophan autofluorescence (excitation at 280 nm, emission filter at 350 nm) on the DISCO beamline.[3,4] Label free imaging, using UV autofluorescence, provides a great tool to follow one single enzyme when acting on a non-UV-fluorescent substrate such as starch. This work describes the first 3D mapping of amylase within single starch granules, in real time and at different hydrolysis times ranging from few minutes to 10 h, without any staining and sectioning. The lateral resolution is also increased when looking in the UV range since it depends directly on the excitation wavelength. This work shows that the same type of starch granules can be degraded in a very different pathway by amylases from different sources and that the starch granules are not degraded at the same time. Thus, this tool is particularly effective for improving knowledge and understanding of enzymatic hydrolysis of solid substrates such as starch and lignocellulosic biomass.

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Molecular Analysis of the *Bacillus subtilis* Minimal NHEJ Repair Complex

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ABSTRACT

Within cells, DNA is constantly exposed to a variety of physical or chemical insults which can damage it. One such injury, the DNA double strand break (DSB) is considered as the most lethal. To counter such damage, cells have evolved two main DSB repair pathways: homologous recombination and non-homologous end joining (NHEJ). The NHEJ is a repair process whereby DNA ends are directly joined together without the need for sequence homology. For a long time NHEJ was thought to be restricted to eukaryotes, recently however a NHEJ pathway was identified in a subset of bacteria, such as the gram positive model *Bacillus subtilis*. Bacterial NHEJ requires only two proteins *in vitro* to perform DSB repair: Ku and LigD. LigD from *B. subtilis* is a multifunctional enzyme displaying ligase and polymerase activities as required depending on the nature of the DNA extremities to be repaired. Bacterial Ku is able to bind DNA ends and stimulates activities of the LigD protein. However, little is known about the DNA binding properties of the bacterial Ku as well as the mechanism used by Ku to stimulate LigD catalytic activities.

B. subtilis Ku is composed of the Ku core in its N-terminal extremity which is homologous to the Ku core of the human Ku70 and Ku86 counterparts. A central and a lysine / arginine rich C-terminal parts extend this Ku core. Our work aims to define the role of these two parts in the bacterial NHEJ mechanism. An *in silico* analysis revealed that more than 95% of the bacterial Ku proteins identified through protein sequence databases mining, display a similar C-terminal extended moiety. The central domain is always present.

By gel shift assay, we showed that Ku is able to form high molecular weight DNA complexes. This property is lost when the C-terminal part of Ku is deleted. Moreover, we showed by electron microscopy analyses that Ku is able to accumulate at the extremities of a linear dsDNA molecule and to spread along this molecule via these ends. This property is increased in the Ku∆Cter mutant. Ku and Ku∆Cter interact with LigD with the same affinity, however Ku better stimulates the ligase activity of LigD than Ku∆Cter.

A mutant of Ku deleted for the C-terminal and the central parts displays the same DNA binding properties as the Ku∆Cter mutant, however it does not stimulate LigD. This Ku-core mutant is no longer able to interact with LigD, demonstrating a role for the conserved central moiety of *B. subtilis* Ku in this interaction. Low resolution SAXS calculated models for Ku and these mutants suggests that the central and the C-terminal parts of Ku are well separated from the conserved ring shape of the human Ku core domain and are thus available to interact with LigD and DNA, respectively.

We propose that the C-terminal part of *B. subtilis* Ku allows to increase the concentration of Ku near DNA ends by limiting its threading capacity along the DNA molecule. This concentration could favour the recruitment of LigD via the central domain of Ku in the vicinity of its substrate: the DNA break. The role attributed by our study to the C-terminal part of the *B. subtilis* Ku protein may represent a well conserved property among all bacterial Ku factors.

Structural Studies of Proteins Inserted in a Half Membrane using SOLEIL Synchrotron Light

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ABSTRACT

Lipids are widely used in human food and animal feed; they also serve as renewable carbon source for biofuels and chemicals. Seeds store the highest levels of lipid concentration in plants, and their overall accumulation mechanisms are well known. Lipids are found in specialized structures called lipid droplets (LDs) which exhibit a unique architecture (a core composed of neutral lipids surrounded by a monolayer of phospholipids) and possess a set of proteins strongly depending on the organism studied. For a long time considered as inert fat depots, LD emerges as an organelle with its own dynamics, capable to synthesize, store and degrade various lipids. LDs from mature seeds contain a limited set of proteins, mostly oleosins. These proteins are unique to plants, and considered as structural and stabilizing proteins. They possess a highly hydrophobic central region (more than 70 amino acid residues) flanked by two hydrophilic N and C terminal domains, and are inserted in the phopholipid monolayer. Oleosin low aqueous solubility is a roadblock to the study of their high resolution structure. Moreover, literature data concerning their insertion in LDs are scarce and those about their secondary structure are contradictory. Oleosins have been maintained soluble and folded in different water soluble objects (from protein/surfactant complexes to purified LDs). A unique coherent set of data for oleosins in both environments: surfactants or native-like yeast LDs was obtained using various biophysical methods most of them based on the use of Synchrotron Radiation. Synchrotron Radiation Circular Dichroism permitted drawing a model in which the hydrophobic central region of oleosins adopts an original β-fold among eukaryotic proteins [1, 2]. OH° radical footprinting is an approach allowing the determination of solvent accessible surfaces [3]. The use of X-rays at Metrology beamline for dissociating water combined with mass spectrometry analysis enables the detection of fine variations of surface exposition resulting from complex formation or conformational rearrangements, at the level of individual amino acid residues.

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PB1-F2 Influenza a Virus Protein Beta Aggregated Secondary Structure: Synchrotron Infrared and Fluorescent Microscopy in Infected-cells

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ABSTRACT

Influenza A viruses (IAV) are responsible every year of seasonal epidemics resulting in considerable illness, death and important economic loss[1]. IAV represent also a major pathogen of animals and cause devastating outbreaks in domestic poultry and massive culling in order to control the spread of the virus. Discovered in 2001, PB1-F2 is an accessory protein of the IAV, considered as a factor of virulence[2]. PB1-F2 is a small protein of 87-90 a.a presenting a strong polymorphism in sequence and length depending on the viral strain[3]. First described as a proapoptotic protein targeting mitochondria, the function of PB1-F2 during the viral cycle is far to be elucidated yet. Recently, several reports described the implication of PB1-F2 in the modulation of innate immune response and the enhancement of the inflammatory response[4,5]. Very little information is available on the structure of PB1-F2. Using recombinant protein, we demonstrated that PB1-F2 has no structure in aqueous solution but is capable to change of conformation depending on the hydrophobicity of the environment from alpha helical conformation to beta sheet secondary structure. Moreover, PB1-F2 has a strong propensity to oligomerizes and form amyloid fibers[6]. PB1-F2 were also detected in IAV-infected cells using thioflavin staining, suggesting a role of the fibers in the pathogenicity of the virus. Thereafter, an electrochemical biosensor was developed to determine the kinetics of expression and oligomerization of PB1-F2 during the viral cycle using specific antibodies[7,8,9]. We evidenced the presence of PB1-F2 soluble oligomers in a time and cell-dependent fashion. However, the putative role of the PB1-F2 beta aggregates (fibers and oligomers) in the virus cycle remains unclear and new techniques were needed to detect them properly in the viral context. In the present study, we tried to evidence the presence of PB1-F2 beta-aggregates in IAV-infected cells at the single cell level using synchrotron radiation. Fourier-transform infrared (FT-IR) and deep UV (DUV) microscopy are non-invasive techniques for monitoring biochemical changes in situ in cells and tissues. Human epithelial pulmonary cells (A549) and monocytic cells (U937) were infected with a wild-type IAV and its PB1-F2 knock-out mutant and harvested at different time post-infection. Infrared spectra were recorded in each condition, then compiled and processed to evaluate the change in the component band of the spectra corresponding to the amide I band (secondary structure changes) and the CH2-CH3 band (membranes and lipids). The data obtained were analyzed by component principal analysis (CPA) and confirmed the presence of an infrared specific beta aggregates signature only in IAV-infected cells expressing PB1-F2. Moreover, the detection of the fibers differs concerning epithelial and monocytic cells in a time-dependent fashion, confirming our previous observation. Taking advantage of the high frequency of tryptophan residues in the sequence of PB1-F2, we were able to correlate the increase of the auto-fluorescent signal recorded by DUV

microscopy with the formation and the accumulation of the beta aggregates in IAV-infected cells. Furthermore, we also observed that PB1-F2 compromise the integrity of the cellular membranes in a cell-type dependent manner. These data should give us further insight into the structure-function relationship of PB1-F2 and to decipher its role in the pathogenicity of the virus.

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Towards a better Understanding of the Cryo-resistance of Lactic Acid Bacteria by using Infrared and UV Synchrotron Radiation

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ABSTRACT

Lactic acid bacteria (LAB) have been used for centuries in the fermentation of meat, vegetables, fruit beverages and dairy products. Scientific evidence of their health benefits, has led to rapid development of probiotic products specially food containing active live cells or nutraceutical dietary supplements. Moreover, some LAB strains can also be used in green chemistry for microbial bioconversion of fuel by-products in producing important industrial-relevant chemicals.

For being commercialized, concentrates of LAB require to be produced by fermentation and stabilized by freezing or freeze-drying. But these stabilization processes induce several types of stresses (thermal, osmotic, mechanical…). These stresses will, in turn, induce cell passive responses involving biophysical mechanisms that may ultimately result in cellular damages. The cell responses and the associated injuries will vary within a cell population leading to an important heterogeneity of cell physiological state.

The challenging objective of this work is the combination of complementary approaches for studying *in situ* and in real time the cellular biophysical response to stresses occurring during the freezing process by probing individual cells or at least small groups of cells.

In this context, UV to infrared photons range emitted by the synchrotron radiation exploited in micro spectroscopy provide a powerful tool for exploring cell composition and properties through *in situ* analysis and mapping with high spatial resolution. We carried out experiments on the infrared beamline SMIS and the DUV fluorescence beamline DISCO at SOLEIL synchrotron. The combined use of the Synchrotron infrared light with high reflective index hemispheres allowed to reach spatial resolution of single cell bacteria. The obtained results made it possible to quantify the heterogeneity of the lipid and protein compositions within a bacterial population[1]. From DUV fluorescence imaging, we obtained first original results on the membrane fluidity of individual cells and on its evolution with temperature[2].

These preliminary works have also identified the need for more sophisticated approaches using these two photons range. In particular, *in situ* analysis of bacteria with infrared spectroscopy calls for dedicated microfluidics devices, accounting from the strong absorption of the surrounding solution. These technological developments are underway and will be reported.

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High-resolution Imaging of Mechanical Properties using a Mid-infrared Synchrotron Radiation

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ABSTRACT

Our general goal is to develop a generic methodology to image the mechanical behavior (rubber or glassy) of structured material based on vibrational spectra of constitutive macromolecules. The concept is illustrated in Figure 1a. It consists in measuring in attenuated total reflection (ATR) mode the local vibrational spectra of the material at different positions. The whole image can be reconstructed either from pencil-beam scanning of the sample (Figure 1a) or from the repetitions of contacts with ATR probe (Figure 1b). The first configuration is preferable for routine measurements, but the second one was used to devise the physical principles. Without any loss of generality, our final intent is to develop a methodology to identify how mechanical constraints propagate during deep frying in potato products. Our test material was accordingly emptied potato parenchyma cell walls (thickness ranging from 1 to 3 µm) equilibrated in super-heated steam prior observations. Measurements were carried out at various temperatures (from 24°C up to 140°C) and various relative humidity (RH from 0.099 to 0.71). These conditions cover a broad range of possible mechanical properties of cell wall constituents with apparent glass transition temperature (T_g) varying from 20°C to 115°C. Vibrational spectra of amorphous (pectins) and semi-crystalline components (cellulose) and mechanical properties are intimately entangled in a composite material. It was thought that the feasibility of the approach could be demonstrated if the displacements of specific bands of the FTIR absorption spectra where similar but opposite when temperature (T) and T_g were shifted. The concept of invariance with T-T_g is indeed a key property of free-volume theory and central in plasticizing theory in polymer systems at thermodynamical equilibrium (without loading). The interpretation of isotherm measurements at different RH and of our temperature scans at constant RH confirmed our initial assumptions. Finally, it is proposed to use a scan temperature to detect regions below and above T_g as shown in Figure 1c. The displacement of COO^- stretching of pectin band (1590-1650 cm^{-1}) offers the best candidate to tailor the observations in parenchyma structures.

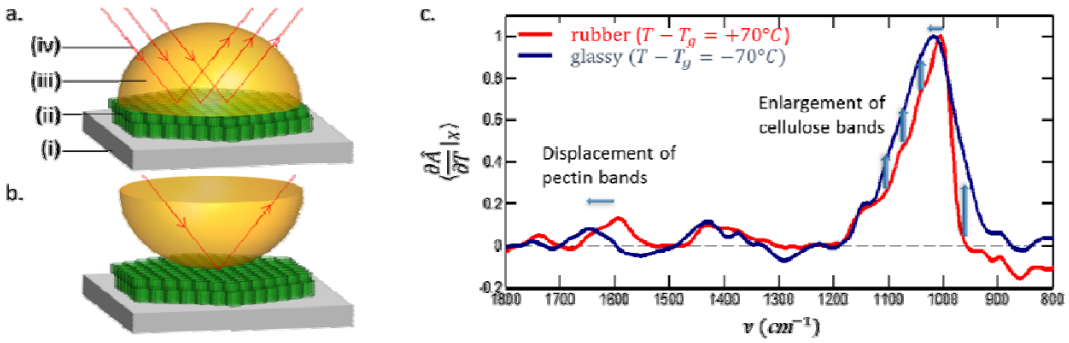

Fig. 1: Principles of mechanical imaging principles: a) using beam scan, b) by displacing the ATR probe, c) dependence of the vibrational spectra of cell-wall constituents with temperature at rubber and glassy states.
i) Heating stage with controlled RH, ii) parenchyma cell walls, iii) ATR ZnSe prism, iv) synchrotron beam.

Astringency and the Interactions between a Human Salivary Proline-rich Protein and Tannins

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ABSTRACT

Astringency is an important organoleptic property of plant-based food. This sensation is due to tannins, which are phenolic compounds. Tannins play an important part in plant defence mechanisms. Indeed, they have an antinutritional effect as they inhibit digestive enzymes. In mammals, the secretion of a family of salivary proteins, called proline-rich proteins (PRPs), has been reported to be link to tannin rich diet. Moreover, PRPs have demonstrated a higher affinity for tannins than other proteins (*1*). Production of salivary PRPs, which belong to the group of intrinsically unstructured proteins (IUP) (*2*), is thus an adaptation process to tannin-rich diets.

The purpose of this work is to provide a closer look at PRP•tannin supramolecular edifices in solution, using a combination of approaches including mass spectrometry (MS), circular dichroism (CD) and Small-angle X-ray scattering (SAXS). These different techniques have been coupled with SOLEIL Synchrotron Radiation (SR).

The human salivary proteins IB5, a basic PRP has been produced by heterologous expression (*2*). After purification, IB5 has been characterized by MS, SAXS and CD (*2-4*). The study of the interaction between IB5 and model tannins by ESI-MS has provided information on the stoichiometries of IB5•tannin non-covalent complexes. SAXS has been used to determine the global structural features of the isolated proteins (*2*) and of the protein•tannin complexes (*5*). Structural studies performed on IB5•tannin edifices by MS/MS experiments (Collision Induced Dissociation, Electron Capture Dissociation and Dissociative Photolonization), by ion mobility spectrometry coupled with MS and by CD showed the presence of a preferential binding site on IB5 (*6, 7*), the nature of the non-covalent interactions (*8*) and conformational changes arising from the interaction (*4*).

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Insights into the Organization of the Respiratory Syncytial Virus Polymerase Complex : Structural Characterization of the N/RNA-P and N^0-P Complexes

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ABSTRACT

Respiratory Syncytial Virus (RSV) is the leading cause of severe respiratory tract infections in young children and calves. Development of antiviral strategies against RSV still constitutes a challenge since there is no human vaccine or antiviral drugs available. The viral polymerase complex (RdRp) (consisting of the four proteins L, P, N, and M2-1) does not present equivalent in the cell and thus represents a target of predilection for drug design. To this end, characterization of the structure and the function of the proteins belonging to the RSV RdRp constitute a prerequisite.

As for all non-segmented negative-strand RNA viruses (*Mononegavirales* order) the genome of RSV is always tightly bound to the viral nucleoprotein (N) and maintained as a helical N:RNA ribonucleoprotein (RNP) complex, which is used as the template for transcription and replication by the viral polymerase. The specific recognition of the RNP by the viral RNA polymerase (L) is mediated by the phosphoprotein (P). During replication of the viral genome, the newly synthesized (+) RNA intermediate antigenomes and (-) RNA genomes must be encapsidated by RNA-free nucleoprotein (N^0). A second function of P is to interact with N and to act as an N-specific chaperone to maintain a pool of N^0. Based on data obtained for Vesicular Stomatitis Virus (VSV) and Nipah virus, the two complexes P-N:RNA and P-N^0 involve different binding sites on both P and N (1,2).

For RSV, the crystal structure of nucleocapsid-like structure was determined (3). However, although N:RNA rings used for 3D structure determination were co-crystallized with P_{CTD} (P binding site on N), no electron densities corresponding to later were observed, and the structure of N:RNA-P complex remains to be characterize. Furthermore, the N°-P complex of the RSV has never been isolated. We have recently generated an N recombinant protein mutated on two residues that are involved in the interaction with RNA. This N protein is i/ monomeric, ii/ RNA-free, and iii/ presents a similar secondary structure than the Nwt, iv/ still interacts with P, suggesting that this mutated N protein could mimic the N^0. Using pull down experiments, we have also shown that the P_{NTD} (residues 1-40) is involved in the interaction with N^0 (4).

The size and flexibility of full-length P render the complexes N:RNA-P and N^0-P complexes unsuitable for X-ray crystallography and NMR study. Determination by SAXS of the radius of gyration of P and N-RNA proteins, and of P-N:RNA and RNA-free N-P(1-40) complexes allowed to obtained important structural insight in the global architecture of these complexes. We expect that, *ab initio* bead models reconstructed from SAXS data, compared to the crystal structure available for N:RNA rings and to the electron microscopy obtained for the complexes, could give information on the binding sites of P on N:RNA and N^0.

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