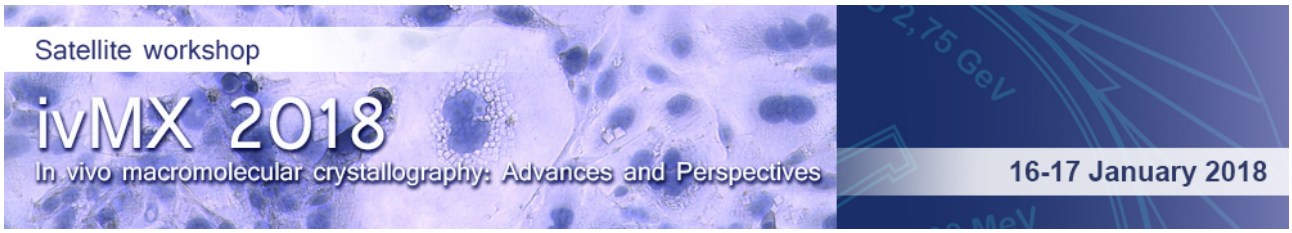

Satellite workshop
"In vivo Macromolecular Crystallography: Advances and Perspectives"

Synchrotron SOLEIL – Saint Aubin, France

16th - 17th January, 2018

BLOCH Auditorium, CEA, l'Orme des Merisiers

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ABSTRACTS BOOKLET

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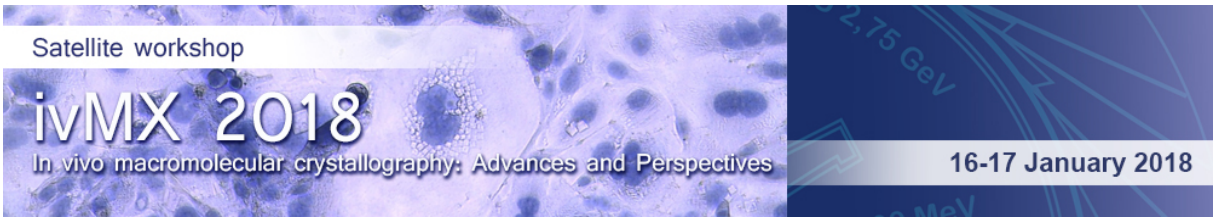
Summary



Welcome!

The workshop on "In vivo Macromolecular Crystallography: Advances and Perspectives" (ivMX) aims to gather the experts and the wider scientific community around the subject of intracellular protein crystallisation. Well-encountered phenomenon for decades, in cellulo crystal appearance grew in interest since its application to the structure determination of macromolecules directly from their natural environment. Recent experimental approaches in favour of a better handling of these complex systems greatly participated in the possibilities in studying the samples with less mechanical stress and sample loss. In this context, new developments in x-ray sources and microfluidics have been the key to the proper opening of these approaches to the wilder scientific community. The crystal growth of macromolecular objects directly from within their natural environment allows to directly link function to structure; with great medical, pharmaceutical and thus societal ramifications.

During this workshop, we will introduce new approaches for in vivo crystal preparation on various types of samples, the methods dedicated for their handling, and all the state-of-the-art strategies for sample characterisation and data collection at various synchrotron-based instruments. Experimental results will be brought forward in lights of new generation x-ray sources, fully related to the scientific needs for structural biology at SOLEIL. New instrumentation and microfluidic developments will be discussed, together with data collection strategies linked to it.

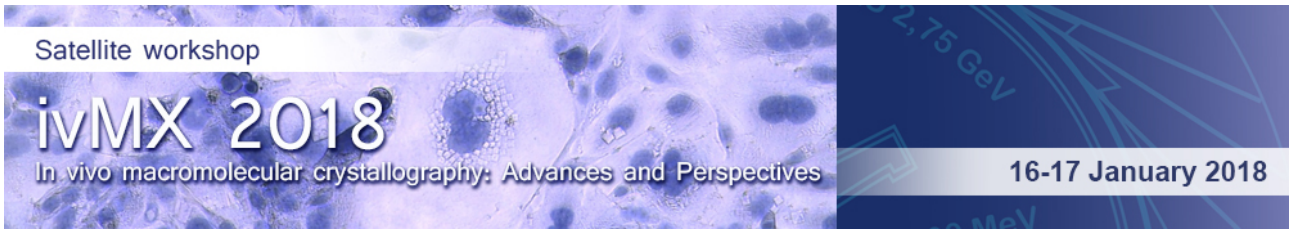
The ivMX 2018 workshop is organised by the Synchrotron SOLEIL, where it will be held as a satellite of the Users' Meeting 2018.

Bienvenue !

Le workshop ivMX "In vivo Macromolecular Crystallography: Advances and Perspectives" a pour but de rassembler la communauté scientifique autour des phénomènes de cristallisation protéique intracellulaire. Phénomène connu depuis des décennies, la croissance de cristaux dans les cellules a fait l'objet d'un intérêt grandissant depuis son application à la résolution de structures de macromolécules dans leur environnement naturel. Grâce aux récents développements expérimentaux, il est désormais possible de manipuler et d'étudier ces systèmes complexes sur des lignes de lumières synchrotrons sans stress mécanique et sans perte d'échantillon. Dans ce contexte, les améliorations dans les sources de rayons x couplées aux développements en microfluidiques ont été des étapes clefs, à l'origine de l'ouverture de cette technique à une plus large communauté scientifique. La croissance des cristaux de molécules biologiques dans leurs environnement naturels permettant de lier fonction et structure, les enjeux sociaux en sont décuplés.

Lors de ce workshop, nous aborderons les approches de production des échantillons cristallins in vivo, les approches adaptées pour leur manipulation, et les nouvelles méthodes de caractérisation et collectes de données structurales. Les résultats expérimentaux seront mis en lumière des développements en cours de réalisation, en liens avec l'expression de besoins scientifiques dans l'optique de l'upgrade de SOLEIL. Le workshop sera également l'occasion de présenter les derniers développements en termes d'instrumentation et de microfluidique

Le workshop ivMX 2018 est organisé par le Synchrotron SOLEIL, où il s'y déroulera comme satellite du Users' Meeting 2018.

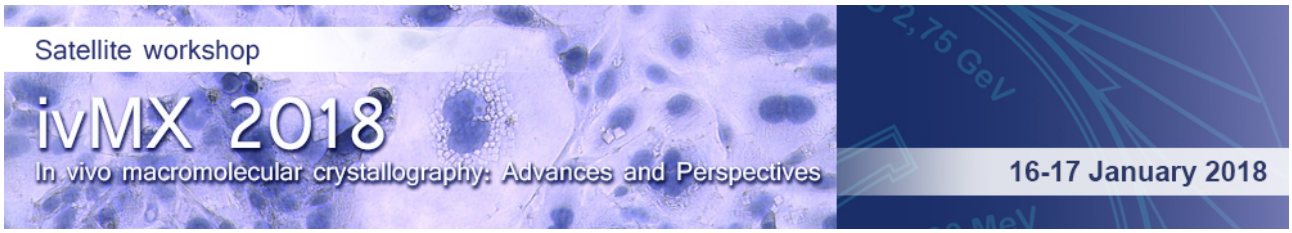


BLOCH Auditorium, CEA, l'Orme des Merisiers

Programme

Tuesday, January 16th

11:30 - 14:00	Registration – Welcome coffee
14:00 - 14:40	**Invited Talk** Setting up an in-vivo crystallography platform at SOLEIL ***Pierre Montaville*** *- Synchrotron SOLEIL, Gif-sur-Yvette, France*
14:40 - 15:20	**Invited Talk** Mammalian crystalline proteins in vivo see the light ***Savvas N. Savvides*** *- VIB-UGent Center for Inflammation Research & Ghent University, Belgium*
15:20 - 15:40	**Short Talk** Lipid membrane architectures promoting ordering and crystallization of proteins ***Angelina Angelova*** *- Institut Galien Paris-Sud, Châtenay-Malabry, France*
15:40 - 16:00	**Short Talk** Pulse-chase experiments to study in vivo crystal formation in a Coccolithophore ***Benedikt Lassalle*** *- Synchrotron SOLEIL, Gif-sur-Yvette, France*
16:00 - 16:40	*Coffee break and group photo*
16:40 - 17:20	**Invited Talk** Structure of a heterogeneous, glycosylated, lipid-bound, in vivo grown protein crystal at atomic resolution ***Subramanian Ramaswamy -*** *Institute for Stem Cell Biology and Regenerative Medicine, Bangalore, India*
17:20 - 18:00	**Invited Talk** In vivo-grown protein microcrystals: The arms and armour of insect viruses ***Fasseli Coulibaly*** *- Monash University, Australia*
19:00 – 21:00	*Aperitif and Dinner at SOLEIL Canteen*



Wednesday, January 17th

08:30 - 09:00	*Coffee break*
09:00 - 09:40	**Invited Talk** Artificial photo-convertible fluorescence proteins: engineering, mechanism, and in vivo crystallization ***Hidekazu Tsutsui*** *- JAIST, Ishikawa & RIKEN, Saitama, Japan*
09:40 - 10:20	**Invited Talk** A potent binary mosquito larvicide revealed by de novo phasing with an X-ray free-electron laser ***Jacques-Philippe Colletier*** *- IBS, France*
10:20 - 11:00	*Coffee break*
11:00 - 11:40	**Invited Talk** Protein crystallography in living insect cells ***Lars Redecke*** *- Institute of Biochemistry Lübeck University & DESY Hamburg, Germany*
11:40 - 12:10	**Invited Talk** Second-order nonlinear imaging: Application in detection and characterisation of proteins crystals ***Frederic Jamme*** *- Synchrotron SOLEIL, Gif-sur-Yvette, France*
12:10 - 12:40	**Invited Talk** Microfluidic positioning of biological macromolecular crystals for serial X-rays diffraction on the Proxima-1 beamline ***Igor Chaussavoine*** *- Synchrotron SOLEIL, Gif-sur-Yvette, France*
12:45 - 14:00	*Lunch at SOLEIL*
14:00 - 15:00	Round table
15:00 - 16:30	Visit of Synchrotron SOLEIL

ABSTRACTS

Satellite workshop
"In vivo Macromolecular Crystallography: Advances and Perspectives"

Tuesday, January 16th

IT-01	Setting up an in-vivo crystallography platform at SOLEIL *P. Montaville*
OC-01	Lipid membrane architectures promoting ordering and crystallization of proteins *A. Angelova*
OC-02	Pulse-chase experiments to study in vivo crystal formation in a Coccolithophore *B. Lassalle*
IT-02	Mammalian crystalline proteins in vivo see the light *S.N. Savvides*
IT-03	Structure of a heterogeneous, glycosylated, lipid-bound, in vivo grown protein crystal at atomic resolution *S. Ramaswamy*
IT-04	In vivo-grown protein microcrystals: The arms and armour of insect viruses *F. Coulibaly*

Setting up an In-vivo Crystallography Platform at SOLEIL

P. Montaville, I. Chaussavoine, F. Jamme, L.M.G. Chavas

Synchrotron SOLEIL, L'Orme des Merisiers, 91190 Saint-Aubin, Gif-sur-Yvette, France

ABSTRACT

The combined use of modern microfocus synchrotron beamlines in conjunction with serial crystallography approaches allows the exploitation of a reasonably low number of micron-sized protein crystals for structure determination. Such micro-crystallography techniques open the door to the investigation of new protein crystallography methods such as the *in vivo* crystallography.

In vivo crystallography is a phenomenon during which naturally or heterologously expressed proteins crystallize within the living cell. This process has been observed to occur in the cytoplasm or within specific subcellular compartments [1]. Such crystals are limited in size but, unlike classical *in vitro* crystallography, are produced in a native-like environment. Anticipated benefits of such system are an alternative way to obtain crystals of proteins for which classical *in vitro* crystal growth revealed unsuccessful as well as the opportunity to study functionally important post-translational modifications. Additionally, *in vivo* crystallography could, when successful, minimize the efforts provided in optimizing purification of samples, currently presenting one of the main bottlenecks of the technique [2].

The investigation of the full potential of *in vivo* crystallography requires an understanding of the parameters underlying the process. The commonly admitted prerequisite is the accumulation of high concentration of the target protein in a restricted location within the living cell. Little is known, however, about the influence of the specific biochemical environment on protein crystal nucleation and growth.

We propose a strategy to explore the possibility to turn cells into elaborated "crystal screens" by addressing multiple protein targets toward specific subcellular compartments (cytoplasm, nucleus, ER, peroxysomes) through a microplate based small scale cell culture setup. In addition, the screens can be further extended by coupling the protein overexpression with the cellular uptake of chemical compounds acting as crystal nucleators such as specific caged lanthanides [3]. The use of such chemical compounds is moreover envisioned to be a way to solve the inherent phase problem of *in vivo* crystallography. Second harmonic generation microscopy (SHG) is a key method to evaluate the "*in vivo* screens", and to get insight of the *in vivo* crystallogenesis process.

The ultimate goal of the project is to build a semi-automated *in vivo* crystallography platform within a synchrotron facility using microfluidics to couple the *in vivo* crystallography screens to the SHG-based scoring step and ultimately to the data collection step through serial crystallography.

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Mammalian Crystalline Proteins *in vivo* See the Light

K. Verstraete[1], E. Persson[1], I. Hendrickx[1], E. Gevaert[2], C. Bachert[2], H. Hammad[1,2], B. N. Lambrecht[1,2], S. N. Savvides[1*]

[1]*VIB-UGent Center for Inflammation Research & Ghent University, Belgium*
[2]*Ghent University Hospital, Belgium*

ABSTRACT

It has been known since 1850 that human tissues harbouring parasitic infections also accumulate elongated hexagonal bi-pyramidal crystals in the extracellular spaces. These crystalline deposits grew to be widely known within the medical community as Charcot-Leyden crystals (CLC) and went on to be described in the lungs of asthmatics and patients with chronic rhinosinusitis. It took another 120 years before these crystals were biochemically defined as protein crystals of human Galectin-10 (also known as CLC protein) and a crystal structure based on recombinant human Galectin-10 ensued just before the turn of the new millennium. At around this time murine YM1, an unrelated protein to Galectin-10, had been shown to display similar auto-crystallization propensities in mice under conditions of inflammation. The structure of YM1 was subsequently determined based on crystals grown using YM1 isolated from murine peritoneal exudate cells.

However, two fundamental questions have remained unanswered: (i) What are the structures of human Galectin-10 and murine YM1 in crystals that grow *in vivo* under conditions of chronic inflammation, and (ii) What are the possible functions of such *in vivo* crystalline proteins.

My presentation will provide some initial answers to these questions as part of an *outside-the-box* research undertaking that unites high-end X-ray crystallography and scattering methods, immunology, and university hospital operation theatres.

Lipid Membrane Architectures Promoting Ordering and Crystallization of Proteins

A. Angelova[1] and B. Angelov[2]

[1]*Institut Galien Paris-Sud, CNRS UMR 8612, Univ. Paris-Sud, Université Paris-Saclay, LabEx LERMIT, F-92290 Châtenay-Malabry cedex, France,*

[2]*Institute of Physics, ELI Beamlines, Academy of Sciences of the Czech Republic, Na Slovance 2, CZ-18221 Prague, Czech Republic*

ABSTRACT

Lipid polymorphism is currently broadly exploited for *in meso* crystallization of transmembrane proteins and peptides. The liquid crystalline structures, formed by hydrated lipids, may be of periodic or of random supramolecular organizations, *e.g.* lamellar, bicontinuous cubic, bicontinuous sponge, and inverted hexagonal phases. Phase transitions may occur in lipid membranous systems upon changes in their composition or in the environmental conditions such as temperature, pressure, hydration level, electrolyte type, ionic strength, or pH of the aqueous medium. The induced curvature changes may strongly affect the protein ordering.

A remarkable similarity exists between the three-dimensional (3D) lipid cubic membranes, which may form in living cells and in synthetic self-assembled systems [1-3]. Confinement of proteins in nanostructured particles provides a means for substantial concentration of the biomacromolecules and for optimal contacts between the neighbouring molecules towards protein ordering and crystallization.

We present time-resolved SAXS investigation of protein molecule entrapment in lipid nanoparticulate containers. Our structural results evidence the stages of the protein loading and ordering in lipid nanoparticles and suggest that protein concentrations higher than 4 mg/ml would be required for biomacromolecules assembly into nuclei for 3D protein crystallization. The obtained structural knowledge from investigations of phase transitions in self-assembled lipid/protein systems may be helpful for the design of experiments on *in-vivo* crystallization of proteins in subcellular compartments.

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Pulse-chase Experiments to Study in vivo Crystal Formation in a Coccolithophore

B. Lassalle

Synchrotron SOLEIL, Saint-Aubin, BP48, Gif-sur-Yvette, France

ABSTRACT

Many organisms form minerals from precursor phases that crystallise under strict biological control. To identify the dynamic intracellular processes of the in vivo crystal formation and its transport remains challenging. The compartmentation of the in vivo nucleation process in the calcifying alga Emiliania huxleyi cells involves differences in Ca and P concentrations, elements that could be traced for clarifying the exact transport and nucleation processes. In the presentation, we will illustrate how pulse-chase experiments could be used with Sr to label the Ca–P-rich phase in E. huxleyi cells, and cryo x-ray absorption spectroscopy together with analytical transmission electron microscopy to follow the Sr within cells. Tracing Sr allowed to clarify the compartimentation of the nucleation and provide further insights in this peculiar in vivo crystallisation.

Structure of a Heterogeneous, Glycosylated, Lipid-bound, in vivo Grown Protein Crystal at Atomic Resolution

S. Banerjee[1], N.P. Coussens[2], F-X. Gallat, N. Sathyanarayanan, K.J. Yagi, J.S.S. Gray, J. Srikanth, S.S. Tobe, B. Stay, L.M.G. Chavas[3,*], S. Ramaswamy[1,*]

[1]*Institute for Stem Cell Biology and Regenerative Medicine, Bellary Road, Bangalore 560065, India.*
[2]*National Center for Advancing Translational Sciences, National Institutes of Health, 9800 Medical Center Drive, Rockville, MD 20850, USA.*
[3] *Synchrotron SOLEIL, 91192 Cedex, France.*

ABSTRACT

in vivo and have been proposed to occur under positive selection pressurein several organisms. Several biological functions that include insulin secretion sorting of secretory proteins in the Golgi apparatus, pathogenesis of *Bacillus thuringiensis*, storage mechanisms for infectious viruses and developmental proteins in seeds and eggs are known. While crystallization of proteins inside cells is well known, we recently reported the first atomic resolution (1.2 Å) structure from *in vivo* grown protein crystals solved by sulfur single-wavelength anomalous dispersion phasing using lower energy X-rays. These are crystals of lipocalin-like milk protein from the pacific beetle cockroach. This protein crystallizes *in vivo* in the midgut of milk-drinking cockroach embryos. These crystals serve as calorie intense complete nutrients for the embryo development. The normal understanding is that *in vitro* crystallization requires highly pure and homogeneous proteins. The structures determined by us reveals a motif that is heterogeneous in protein sequence, glycosylation as well as the bound lipid. *In vitro* expression and secretion of the milk protein in yeast is in progress.

Recently we have also observed similar crystals in the growing stages of the fruit fly. We are now in the processes of isolating these crystals and characterizing them. The talk will present the published results as well as work done after that.

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In Vivo-grown Protein Microcrystals: The Arms and Armour of Insect Viruses

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ABSTRACT

While in vitro protein crystallization has been studied for decades to facilitate the production of crystals for structural analysis, ubiquitous insect viruses have evolved proteins that readily crystallize *in vivo* despite the complexity of the cellular environment. Such viral crystals are among the most striking examples of protein self-assembly, leading to the formation of ultra-stable microcrystals filling most of the infected cells.

These crystals were initially characterised as infectious micro-capsules that package up to hundreds of virus particles. The crystalline matrix forms an armour that allows the virus to persist for years in the environment like bacterial spores. In addition, insect poxviruses also produce a second type of *in vivo* crystals called spindles that do not contain virus particles but strongly enhance the virulence of these pathogens. Interestingly, administration of spindles can also potentiate the effect of unrelated viruses and toxins.

Over the last few years, we have developed X-ray microcrystallography methods allowing structure determination of these different *in vivo* crystals directly purified from infected insects. This presentation will compare the molecular organisation and specific characteristics of the four classes of viral, *in vivo* crystals known to date and present a pipeline for their efficient structural analysis in cellulo.

Satellite workshop
"In vivo Macromolecular Crystallography: Advances and Perspectives"

Wednesday, January 17th

IT-05	Artificial photo-convertible fluorescence proteins: engineering, mechanism and in vivo crystallization *H. Tsutsui*
IT-06	A potent binary mosquito larvicide revealed by de novo phasing with an X-ray free-electron laser *J-P. Colletier*
IT-07	Protein crystallography in living insect cells *L. Redecke*
IT-08	Second-order nonlinear imaging: Application in detection and characterisation of proteins crystals *F. Jamme*
IT-09	Microfluidic positioning of biological macromolecular crystals for serial X-rays diffraction on the Proxima-1 beamline *I. Chaussavoine*

Artificial Photo-convertible Fluorescence Proteins: Engineering, Mechanism, and in vivo Crystallization

H. Tsutsui[1,2]

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[2] *Brain Science Institute, RIKEN, Saitama 351-0198, Japan*
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ABSTRACT

"Kaede" is a natural photoconvertible fluorescent protein, first discovered from the coral Trachyphyllia geoffroyi (1). It contains a tripeptide, His-Tyr-Gly, which acts as a green chromophore that is photoconvertible to red following UV irradiation. We have worked on engineering of artificial optical highlighter from non-photoconvertible natural fluorescent protein basing on in vitro molecular evolution strategy (2). Through such efforts, we have developed efficient optical highlighters (2, 3), revealed chromophore photoreactions during color conversion (4), and furthermore, encountered with a variant which spontaneously forms into a crystal in mammalian cells (5). In this talk, I like to share our findings, observations, as well as future perspectives.

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(4) Tsutsui et al., Chem. Biol. 16, 1140-1147, 2009
(5) Tsutsui et al., Mol Cell, 58, 186-193, 2015

A Potent Binary Mosquito Larvicide Revealed by *de novo* Phasing with an X-ray Free-electron Laser

J-P. Colletier[1], M.R. Sawaya[2,3], M. Gingery[2], J.A. Rodriguez[2], D. Cascio[2], A.S. Brewster[4], T. Michels-Clark[4], N. Coquelle[1], S. Boutet[5], R.H. Hice[6], G.J. Williams[5], M. Messerschmidt[5], D.P. DePonte[5], R.G. Sierra[5], H. Laksmono[5], J.E. Koglin[5], M.S. Hunter[5], H-W. Park[6,7], M. Uervirojnangkoorn[8], D.K. Bideshi[6,7], A.T. Brunger[8], B.A. Federici[6], N.K. Sauter[4], D.S. Eisenberg[2,3]

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ABSTRACT

We will report on the first macromolecular crystal structure ever to be determined from crystals so small that they measure only fifty unit cells on edge. The crystals are naturally occurring, and were thus produced *in vivo*.

At 2.25 Å resolution, this structure marks an advance toward the dream of determining atomic resolution structures from even smaller, and more complex naturally occurring objects, such as organelles. At 92 kDa, the crystallographic asymmetric unit is nearly three times larger than any previously phased *de novo* using XFEL diffraction and serial femtosecond crystallography (SFX). Our use of multiple heavy atom derivatives for *de novo* phasing offers encouragement for investigators seeking structures for which no structure exists of a known homolog.

This structure sheds light on BinAB, a naturally occurring mosquito toxin produced and distributed in crystalline form worldwide to combat the devastating diseases borne by mosquitos, including malaria, yellow fever, dengue, encephalitis, and filariasis. We reveal the extensive intermolecular interactions that maintain the potency of the binary toxin, the mechanism for its pH triggered release, internalization in the mosquito larvae gut cell, and the basis for structural transformation into a membrane-spanning pore.

Protein Crystallography in Living Insect Cells

J.M. Rudolph[1,2], R. Schönherr[1], W.M. Riekehr[1], J. Kaiser[1], C. Klapproth[1], G. Bourenkov[3], T.R. Schneider[3], A. Meents[2] and L. Redecke[1,4]

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ABSTRACT

Protein crystallization in living cells has been observed in all domains of life. This crystallization approach holds the possibility to grow a huge number of micron-sized protein crystals with comparable properties and of high quality [1-3]. To systematically exploit the potential of *in cellulo* crystallization in living insect cells for structural biology we streamlined this process by establishing a pipeline to elucidate the structural information of *in cellulo* crystallized target proteins in short time. After cloning of the target gene into baculovirus transfer vectors, the associated recombinant baculoviruses are generated to infect insect cells, and crystal formation is detected at day 4 to 6 after infection. If intracellular crystallization is successful, diffraction data of the isolated *in cellulo* crystals are collected using serial crystallography approaches at XFELs [4] or highly brilliant synchrotron sources [5], depending on the size of the obtained crystals. In our hands, these efforts resulted in the successful crystallization of more than 20 different proteins in living insect cells so far. However, two major bottlenecks currently restrict a more broad application: Depending on the recombinant protein, the number of crystal containing cells varies between more than 70 % and less than 1 %, and changes of environmental conditions during cell lysis and crystal purification result in a loss of crystal quality.

To overcome these limitations we recently established techniques for serial diffraction data collection from *in cellulo* grown crystals directly within living cells using synchrotron and XFEL radiation, combined with intracellular labelling of the recombinant target proteins with heavy metals. These innovative approaches avoid crystal purification and transfer of the living, crystal-containing cells, allow direct screening of cell cultures for successful *in cellulo* protein crystallization using the X-ray beam, and will gain access to direct phasing methods. Thus, our current *in cellulo* crystallization pipeline will be further improved to elucidate structures of proteins without prior information, and limitations in data collection due to low intracellular crystallization efficiency will be overcome, supported by additional integration of fluorescence-based cell sorting. Our results pave the way to more efficiently use crystal containing cells as suitable targets for serial diffraction data collection at synchrotrons and XFELs in the future.

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Second-order Nonlinear Imaging: Application in Detection and Characterisation of Proteins Crystals

P. Montaville, F. Jamme

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ABSTRACT

Second-order nonlinear imaging such as second harmonic generation (SHG) microscopy is a powerful technique for crystals imaging and characterization. The SHG signal is proportional to the square of the applied laser intensity and occurs at the focus of the microscope objective. As molecules approach isotropic ordering, the coherent SHG signal generated from the ensemble reaches zero due to internal cancellation. However, when molecules become more ordered, the SHG signal from individual proteins constructively adds. SHG is strictly forbidden in centrosymmetric space group but the chiral nature of proteins crystals results in crystals adopting non-centrosymmetric space groups . Thus, SHG microscopy is an emerging technique for imaging highly ordered protein crystal detection, and revealed specifically useful for in-vivo crystallography when small sized crystals are produced in living cells. Nonlinear optical (NLO) imaging involve the response of the biological material to an incident laser beam, and emission of the induced new optical wave carries signatures of the imaged material. Therefore, the nature of the nonlinear interaction (as polarization and emission directionality) that provides a powerful imaging contrast for proteins crystal characterisation will be presented and discussed.[1–6]

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Microfluidic Positioning of Biological Macromolecular Crystals for Serial X-rays Diffraction on the Proxima-1 Beamline

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ABSTRACT

One of the main recent evolution in macromolecular crystallography is the implementation of serial crystallography at hard x-ray Free Electron Lasers and synchrotrons, allowing to further expand the spectre and possibilities in crystallography. The techniques validated to date bring successful results, yet require extensive amounts of sample crystals and recorded images to start structure analysis; potentially, this also require long beamtime shifts at x-ray FELs. At various synchrotron sources including SOLEIL, serial crystallography was adapted by using microfluidic chips[1] to handle batches of macromolecular crystals and expose those to the synchrotron beam. These new developments allow acquiring diffraction data over several degrees per crystal, permitting to drop by several orders of magnitudes the number of crystals required for acquiring a complete data set. In addition of facilitating a better control during serial crystallography experiments, microfluidic chips provide with the possibility of mastering the crystal environment, the injection of ligands, or even opens access to diffraction experiments on biologically hazardous molecules. Moreover, the trapping method employed within the microfluidic chips facilitates bypassing most of the chemical issues currently occurring when handling and preparing macromolecular crystals for diffraction experiments. Coupled with a proper design, diverse kind of molecules could be trapped, including cells used for *in cellulo* crystallography.

The presentation will show the results of microfluidic induced macromolecular serial crystallography at synchrotron SOLEIL. Using specific patterns inspired from an experiment by Lyubimov[2], macromolecular crystals or cells are trapped at known and precise positions within a microfluidic chip. With , The chip can be handled and oriented using a 3D-printed frame placed on the goniometer at PROXIMA-1[3] beamline.The first results illustrated here were obtained using an *in house* device; complete data could be acquired with high redundancy prior to solving the structure of a test protein.

REFERENCES

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