BSI@Paris-Saclay

21/11/2024

ENS Paris-Saclay - LBPA

Abstracts

Oral presentations and posters

08h30-09h00 **Welcome**

09h00-09h30 **Introduction**

Session I

09h30-09h50 **Clarisse Fourel** (IBPC, ICSN), Unraveling the intimate coupling between lipid and scaffold protein dynamics in nanodiscs by high pressure NMR.

09h50-10h10 **Armelle Gesnik** (I2BC), Structural study of gene rearrangement mechanisms driven by transposases in collaboration with the c-nhej pathway in paramecium tetraurelia

10h10-10h30 **James Provan** (I2BC), The first structural observations of an XerCD-DNA recombinase complex

10h30-11h00 Coffee break and posters

11h00-12h00 **Magali Noiray** (Plateform PIM, I2BC)**, Julie Menetrey** (Frisbi), **Stéphane Bressanelli** (CryoEM Soleil/I2BC)

12h00-14h00 Lunch and Posters

14h00-15h00 Invited Speaker: **Clément Charenton** (IGBMC) – Title to be Announced

Session II

15h00-15h20 **Marie-Hélène Le Du**, (I2BC) BioStIn-E : un site d'autoformation à la biologie structurale intégrative

15h20-15h40 **Thibault Tubiana** (I2BC), Deciphering viral protein-protein interactions using AlphaFold.

15h40-16h00 **Damila Mihovilcevic** (I2BC), Incorporation of lanthanides in the sensor/transducer MecR1 protein of Staphylococcus aureus to monitor conformational changes

16h00-16h30 Coffee break and posters

16h30-17h30 Overall discussion with BSI community

ORAL PRESENTATIONS

Unraveling the intimate coupling between lipid and scaffold protein dynamics in nanodiscs by high pressure NMR.

Clarisse Fourel*[1,2], Alexandre Pozza[1], François Giraud[2], Elodie Point-Bonnet[1], Christel Le Bon[1], Karine Moncoq[1], Laurent Catoire[1], and Ewen Lescop[2]

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Résumé

Biological membranes play a fundamental role in cell function, ensuring molecular and signal passage and regulating processes such as cell signaling. Nanodiscs (NDs) are a widely used model for mimicking biological membranes and studying membrane proteins in a lipid environment. These _˜10nm nanoparticles consist of hundreds of phospholipids surrounded by lipoproteins. In the literature, phospholipid-lipoprotein interactions in NDs are not well characterized. Because NDs are very convenient and highly used tools for providing a simplified bilayer lipid environment for membrane proteins, we aimed at improving our knowledge of the mutual impact of the lipoprotein structure and dynamics and the lipid phase transition in NDs.
To reach these objectives, we use hydrostatic pressure and temperature as tools to modulate lipid dynamics. We mostly used liquid-state NMR spectroscopy as a technique capable of revealing atomic-resolution dynamic details simultaneously for lipid and lipoprotein and by taking advantage of the high-pressure apparatus at ICSN.

We have recently shown (Pozza et al., Nature Comm, 2022) by 1H NMR that ND formed from D1 lipoprotein undergo detectable gel/fluid transitions under high pressure. We further showed from 13C chemical analysis that the fluid-> gel transition is associated, as expected, by an increased population of the *trans* conformers in the hydrophobic tail. To further assess the impact of the size of the NDs, we prepared NDs using the lipoproteins D1 (reference, 10nm), E3 (larger, 12nm) and DH5 (smaller, 8nm). Using 1H NMR intensity analysis at various temperature and pressure values, we show that the size of the ND has a strong effect on the DMPC lipid phase transition (value of the transition pressure Pm). This suggests that lipid confinement and/or the presence of the scaffold protein alter the local lipid phase diagram.
To assess the behavior of the lipoprotein during the lipid phase transition, we also monitored a 15N, 13C and partially 2H labelled lipoprotein D1 within a DMPC ND by NMR. The 15N and 13C HSQC spectra revealed significant side chain orientation for a subset of tryptophan and asparagine/glutamine residues, most likely associated with the partial exclusion of the lipoprotein from the gel phase. Unexpectedly, we also observed a transient conformational state of the lipoprotein after a rapid cold shock. This transient conformational state with

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a _˜hours lifetime remains to be structurally characterized. This highlights the complex energy conformational landscape of NDs in terms of thermodynamic and kinetic behaviors, that needs to be considered during their manipulation and further explored.

STRUCTURAL STUDY OF GENE REARRANGEMENT MECHANISMS DRIVEN BY TRANSPOSASES IN COLLABORATION WITH THE C-NHEJ PATHWAY IN PARAMECIUM TETRAURELIA

Armelle Gesnik*[1], Joël Acker[1], Julien Bischerour[1], Sonia Baconnais[2], Mireille Bétermier[1], Virginie Ropars[1], and Jean-Baptiste Charbonnier[1]

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Résumé

DNA double-strand breaks (DSBs) are genotoxic lesions that threaten the integrity of the genome. These breaks are repaired either by homologous recombination (HR) or by the non-homologous end-joining pathway (NHEJ) (1). In the classical NHEJ pathway, the ring-shaped Ku70/Ku80 (Ku) heterodimer binds to the damaged DNA ends, facilitating the recruitment of the ligation complex to repair the broken ends. Although potentially dangerous, programmed DSBs (prDSBs) are essential for a number of physiological processes. In *Paramecium*, the PiggyMac (Pgm) transposase and its partners, the PiggyMac-Like (PgmL) proteins precisely excise Internal Eliminated Sequences (IES) during the differentiation of the new macronucleus (2)(3). A transposase domain has been predicted in all five PgmL proteins, from PgmL1 to PgmL5 but they lack the DDD catalytic triad. Therefore, their role in prDSB seems to be architectural to form an endonuclease complex with Pgm (3). The prDSBs generated by Pgm and PgmL at IES sites are subsequently repaired by the NHEJ pathway, highlighting the importance of a close association between DNA cleavage and DSB repair (4). Moreover, *Paramecium Tetraurelia* exhibits several homologs of Ku70 and Ku80, capable of forming different heterodimers of Ku. However, only the Ku70a/Ku80c homologs are implicated in this pathway of programmed double-strand break repair.
Our research focuses on unravelling the unknown interaction mechanisms of various factors involved in the process. Specifically, we aim to comprehend how the endonuclease complex forms and binds to the Ku heterodimer. Additionally, we seek to address the question of why only the Ku70a/Ku80c heterodimer is implicated in prDSBs, while the Ku70a/Ku80a heterodimer does not participate in this process. To start the investigations, I successfully expressed the PiggyMac interactome in insect cells, using the MultiBac system implemented in the laboratory. Then, I optimized the purification protocol of Pgm, PgmL1, PgmL3 and Ku. To gain further insights, I conducted preliminary interaction tests for PgmL3 using a non-specific DNA substrate, employing gel shift assays. Recently, I initiated the structural

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study of PgmL1 by negative-stained TEM, as well as the cryoEM structural study of the Ku heterodimer and PgmL3. These research endeavours will contribute to a comprehensive understanding of the intricate processes at play during prDSBs and their subsequent repair mechanisms.

1. Lieber MR. The Mechanism of Double-Strand DNA Break Repair by the Nonhomologous DNA End-Joining Pathway. Annu Rev Biochem. 7 juin 2010;79(1):181-211.

2. Baudry C, Malinsky S, Restituito M, Kapusta A, Rosa S, Meyer E, et al. PiggyMac, a domesticated *piggyBac* transposase involved in programmed genome rearrangements in the ciliate *Paramecium tetraurelia*. Genes Dev. 1 nov 2009;23(21):2478-83.

3. Bischerour J, Bhullar S, Wilkes CD, Mathy N, Dubois E, Singh A, et al. Six domesticated PiggyBac transposases together carry out programmed DNA elimination in Paramecium. :24.

4. Bétermier M, Borde V, de Villartay JP. Coupling DNA Damage and Repair: an Essential Safeguard during Programmed DNA Double-Strand Breaks? Trends in Cell Biology. févr 2020;30(2):87-96.

The first structural observations of an XerCD-DNA recombinase complex

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Résumé

Xer recombination is a ubiquitous process which enables the resolution of bacterial chromosome dimers at cell division. The Xer system of most bacterial species is composed of two related proteins, XerC and XerD, which are part of the wider tyrosine recombinase family with well-known enzymes such as Cre and Flp. An active Xer recombinase complex is heterotetrameric, containing two XerC units, two XerD units, and two DNA duplexes, which are subsequently recombined through a Holliday-Junction intermediate.
The Xer system is also commonly hijacked by mobile genetic elements to drive their integration into a host chromosome. These elements are termed IMEX (Integrative Mobile Elements exploiting Xer) and have evolved diverse specialized accessory proteins and/or accessory DNA sequences to circumvent the strict spatio-temporal control of typical Xer recombination. The IMEX bacteriophages of *Vibrio cholerae* are central to the transmission of the pandemic disease Cholera, as the Cholera toxin genes are encoded within the phage CTXΦ genome.

Structural studies of XerCD have been attempted without success since the discovery of the system over 30 years ago. Practical difficulties surrounded the highly recalcitrant nature of XerC, with problems of purification, solubility, and aggregation. Our recent work has developed simple methodological advances that have enabled the purification of homogeneous *V. cholerae* XerCD-DNA complexes at high concentrations. We believe our new methods should be applicable to a wide array of insoluble DNA-binding proteins.
For the first time we have been able to produce XerCD-DNA crystal structures and cryo-EM reconstructions, and we have begun to answer biological questions about assembly, interactions, and regulation of the recombinase complex. Through specific DNA substrates, and/or the use of certain protein mutants, we can force the complex to adopt different conformational states to gain specific insights. In combination with our long-running molecular-genetic research into Xer, this new structural viewpoint will shed light on how this system is hijacked by IMEX such as *V. cholerae* CTXΦ. In particular we wish to understand why certain closely related bacterial species may have, or may lack, IMEX.

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BioStIn-E : un site d'autoformation à la biologie structurale ihntégrative

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Résumé

Le développement accéléré de la biologie structurale intégrative dans les laboratoires de biologie nécessite un niveau de compétence élevé qui doit s'acquérir de plus en plus rapidement et couvre les disciplines de la biochimie, chimie, physique, mathématique et science de l'informatique. Les niveaux peuvent facilement rester très (trop) superficiels dans chacune des disciplines concernées, ce qui augmente le risque de résultat erroné, ou les pertes de temps liées à des choix erronés.
Après plusieurs sessions du MOOC "Voyage au coeur du vivant avec des rayons X : la cristallographie", j'ai constaté deux limites à cette approche, en particulier quand il s'agit d'initier des étudiants qui seront nos futurs structuralistes : la contrainte du temps, liée à la période de diffusion du MOOC ; la contrainte du contenu, qui reste classique dans le sens ou on présente un contenu unique tout au long de ce MOOC.

Pour essayer de pallier à ces limites, je coordonne la préparation d'un site web d'autoformation à la biologie structurale intégrative. Il s'agit d'une initiation 'à la carte' dans laquelle, pour chaque discipline concernée, l'étudiant commence par évaluer ses connaissances en complétant un quiz, puis, selon le résultat au quiz, il pourra suivre une vidéo de formation ou passer à la suite.
Au cours de ma présentation, nous parcourrons le site web, et je vous proposerai de tester ensemble vos connaissances dans un domaine particulier de la biologie structurale intégrative.

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Résumé

In eukaryotes, the spliceosome removes non-coding introns from precursor messenger RNAs during pre-mRNA splicing. Crucially, many pre-mRNAs are spliced differently depending on cellular status or external stimuli. This "alternative splicing" reshapes the genetic information from a given mRNA to encode several protein isoforms and greatly diversifies proteomes. Splicing must be extremely precise as errors produce aberrant mRNA encoding potentially toxic proteins. Splicing fidelity relies on the accuracy of the spliceosome that assembles de novo on each pre-mRNA in a cotranscriptional manner, gets activated and selects the precise boundaries of introns to catalyse their excision. The spliceosome is endowed with two apparently conflicting properties: it must be very accurate to avoid splicing errors while being tolerant to accommodate alternative splicing changes.
Our laboratory uses a combination of biochemistry and structural biology (cryo-EM) to reveal the molecular organization of splicing complexes captured during key fidelity checkpoints. In my presentation, I will discuss our research on spliceosome activation, highlighting its implications for splicing fidelity and human neurodevelopmental disorders.

Deciphering viral protein-protein interactions using AlphaFold.

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Résumé

In recent years, the field of structural biology has undergone significant transformations, with the advent of revolutionary technologies and methodologies. A notable milestone in this journey is the development of AlphaFold, a cutting-edge software capable of accurately predicting the three-dimensional structures of proteins based solely on their amino acid sequences. This tool has also shown remarkable proficiency in forecasting the interactions between different proteins. However, a notable omission in the vast repository of models in the AlphaFold database is the absence of viral proteins. This gap presents a unique opportunity for researchers to delve into the modelling of viral protein structures independently.
We have recently used AlphaFold to reliably study the domain decomposition and structural organization of the hepatitis E virus ORF1p protein (Fieulaine et al. 2023). Building on this success, we have extended our research to investigate the interactions between the hepatitis E virus capsid protein (ORF2i) and various cellular partners. We have employed a methodical "Top-Down" approach in our modelling. This process starts with the modelling of entire systems to pinpoint potential interactions. After identifying these interactions, we then refine our models to concentrate solely on the key areas where these interactions occur.

By applying this methodology, we were able to identify a potential novel dimerization area in the NS5A protein of the Hepatitis C virus. This discovery was further studied and validated through molecular dynamics simulations and biophysical measurements including Isothermal Titration Calorimetry (ITC), differential scanning fluorimetry (nanoDSF) and nuclear magnetic resonance (NMR).
Our work shows how versatile AlphaFold is for studying viral proteins and its potential to reveal new details about how these proteins behave and interact.

*Intervenant

Incorporation of lanthanides in the sensor/transducer MecR1 protein of Staphylococcus aureus to monitor conformational changes

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Résumé

Methicillin resistant *Staphylococcus aureus* (MRSA) is a pathogen that poses a worldwide threat. MRSA has an integral membrane metalloprotease protein, MecR1, that is activated by the covalent binding of β-lactam antibiotics to the extracellular sensor domain. Activated MecR1 would cleave the cytoplasmatic repressor protein MecI, resulting in the expression of the resistance determinants. Cryo-EM structures of BlaR1, homologous of MecR1, revealed the global structure of these sensor/transducer proteins but, gave little insights on how the metalloprotease domain is activated upon β-lactam biding. Here we present an EPR strategy for monitoring β-lactam-induced conformational changes in MecR1.
We have established an *E. coli* expression protocol to purified MecR1 in detergent micelles. SEC-MALS measurements showed that this recombinant protein existed in a monomer and dimer equilibrium. Using the fluorescent penicillin analogue BocillinTM FL we confirmed that the sensor domain of the purified protein remains functional. Since MecR1 has seven cysteines, conventional nitroxide-based site directed spin labelling was expected to be difficult. Instead, we decided to engineer lanthanide binding tags (LBT) that bind Gd(III) with high affinity onto MecR1, to monitor conformational changes and study its oligomerization state using EPR.

Luminescence experiments demonstrated that the Tb(III) binds to the engineered LBT as intended. The 94 GHz Gd(III) EPR spectra of MecR1 with Gd(III):LBT spin labels exhibited shoulders indicative of large zero-field interactions similar to those previously reported. Surprisingly, we found that the wild-type MecR1 (MecR1-WT) that did not have LBT, also binds Gd(III). Its EPR spectrum lacked these shoulders. One likely position is the native Zn-site of MecR1. Many Zn-proteases are able to bind different metal ions.

We carried out 94 GHz RIDME (Relaxation Induced Dipolar Modulation Enhancement) measurements on the Gd(III) labelled MecR1. Tikhonov analysis of the doubly labelled RIDME traces yielded a distance distribution with a maximum at 2.9 nm, similar to the expected intramolecular LBT:Gd(III)-LBT:Gd(III) distance based on the BlaR1 structure.

*Intervenant

POSTERS

Biochemical characterization of inhibitors for the histone chaperone ASF1

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Résumé

In eukaryotic cells, the genetic information is stored in DNA, organized into chromatin, which elementary unit is the nucleosome, a wrap of 147 base pairs around an octamer of histones. Histones chaperones are paramount for DNA replication, both for preserving genome integrity and contributing to nucleosome dynamic. The histone chaperone ASF1 (Anti-silencing Function-1) is involved in nucleosome assembly/disassembly and regulation of gene expression. Its overexpression is correlated to tumor proliferation, in particular for triple-negative breast cancer1. On the other hand, its knockdown makes cancer cells more sensitive to chemo- and radiotherapy.

These properties make ASF1 an interesting therapeutic agent and have motivated the design of peptide inhibitors for specific target. These peptides have 2 epitopes to mimic the interaction of ASF1 with H3-H4, bound with a linker. A first generation of high-affinity peptides have been designed2, which showed selective association with ASF1 in vitro and good penetration when combined with a Cell Penetrating Peptide (CPP). Their sensibility to proteases motivated the design of a new generation of peptide-like molecules called foldamer with a urea backbone3, which prevents their degradation by the proteasome. Previous work optimized the design of foldamers for their affinity for ASF1 with modified residues both with a long linker and with a short linker4. The best candidates for both categories showed nanomolar affinity with ASF1.

Our team is currently working on further characterizations of these foldamers to compare them to the first-generation CPP-peptides. First, the in-cellular penetration of foldamers has been determined with fluorescent compounds and confocal microscopy. Then, the biological effect of the compounds has been assessed: their effect on cellular proliferation and toxicity is measured by epifluorescence and the effect on the cell cycle is evaluated by an EdU assay. Finally, the ability of the peptide/foldamer to bind specifically to ASF1 in cellular extract has been evaluated by pull-down with biotinylated compounds.

*Intervenant

Structural characterization of WRN helicase Ku-binding motif in the context of precision oncology

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Résumé

Precision oncology seeks to target cancer cells without targeting healthy cells, unlike conventional chemo- and radiotherapy approaches. Recent studies have shown that microsatellite instability (MSI) cancers with a defect in the mismatch repair (MMR) pathway are dependent on the Werner syndrome helicase (WRN) for their survival. WRN is a member of the conserved RecQ helicase family, important for genome integrity in human. Recent studies have targeted the inhibition of WRN helicase activity with a number of promising strategies, but a major difficulty lies in the specific targeting of WRN compared to other RecQ helicases, as the helicase active site is well conserved between proteins of this family.
One innovative approach is to target the interaction of WRN with its main partner in the nucleus, the Ku70/80 (Ku) heterodimer. WRN interacts with Ku via Ku-binding motifs (KBMs) present at the N- and C-termini of the protein. We have shown elsewhere that inhibition of this interaction disrupts the recruitment of WRN at DSB induced after laser micro-irradiation and subsequently induces WRN degradation.
I will present how we managed to select 20 inhibitors of the Ku-WRN interaction and the first structural and biophysical data on these inhibitors. I will also present results on the characterization of the KBM C-terminus of WRN in complex with Ku by X-ray crystallography and cryo-EM approaches.

*Intervenant

Characterization of the phospho-dependent interactions between Mdm2 and p53.

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Résumé

The E3-ubiquitin ligase Mdm2 is the main negative regulator of the "tumor suppressor" p53. This Mdm2-p53 axis is altered in nearly all cancers and is thus an attractive anti-cancer target, but the current inhibitors have clinical limitations.

Mdm2 is phosphorylated by two classes of kinases: i) under normal conditions, Ck1δ phosphorylates the Intrinsic Disorder Region 1 (IDR1) of Mdm2, whereas ii) in response to DNA damage, ATM/ATR/DNAPK kinases are activated and phosphorylate the IDR2 of Mdm2. So far, all the biochemical/biophysical works on Mdm2-p53 have been achieved using non-phospho, i.e. non-native, fragments of these proteins.

We sought to characterize the hyperphosphorylated forms of Mdm2. We have produced recombinantly a number of fragments containing IDR1 and/or IDR2, and the full-length protein. In their 13C/15N labeled version, we used NMR spectroscopy to monitor their phosphorylation in a site-specific fashion. We identified 14 phosphorylation sites on IDR1, 8 on IDR2, and determined the corresponding phosphorylation mechanisms. Then, we have characterized the NMR relaxation and the intramolecular interactions between the 3 folded domains of Mdm2 and IDR1/2, in the non-modified and in the phosphorylated forms. These reveal weak interdomain interactions, which might be exploited to tune Mdm2 activity. Moreover, these have consequences on the interaction with p53, which we quantified using ITC on a multitude of constructs. Hence, we present a pioneer structural study on hyperphosphorylated IDRs, a common object in eukaryotic cells but much less in the literature.

*Intervenant

Development of Inhibitors for the Histone Chaperone ASF1

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Résumé

Histones are essential in the epigenetic regulation of gene expression and DNA accessibility. During dynamic processes such as DNA replication, chromatin undergoes specific changes that can lead to a loss of histone-related information, resulting in diseases like cancer. ASF1, a histone chaperone, is crucial for nucleosome assembly/disassembly and gene expression regulation. ASF1's overexpression is particularly associated with aggressive cancer types, such as triple-negative breast cancer. Conversely, ASF1 knockdown has been shown to sensitize cancer cells to chemotherapy and radiotherapy, positioning ASF1 as a promising target for therapeutic intervention. Thus, ASF1 has emerged as a promising therapeutic target.
This project aims to develop competitive inhibitors for ASF1 by disrupting its interaction with histones. Initial designs use competitive peptides, yet these are highly susceptible to protease degradation, necessitating further optimization. Two main strategies are being explored: (1) the development of peptidomimetic structures with ureas, which are more resistant to proteases, as well as have similar properties, and (2) the use of "stapled" peptides to stabilize the helical conformation and increase binding affinity. Together, these approaches aim to create robust and selective inhibitors of ASF1, marking significant progress toward novel cancer therapies that target histone chaperone functions.

These unnatural peptides are poorly managed by software and need specific treatment and additional files to consider them as a polymer, not a unique molecule.
These approaches represent key steps toward therapeutic inhibitors for targeting ASF1 in oncology.

*Intervenant

Higher-order multimerisation of a ubiquitin E3 ligase: the case of SIAH1

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Résumé

E3 ubiquitin ligases promote ubiquitination by stabilising an active complex between a loaded E2 conjugating enzyme and a substrate protein. To fulfill this function, SIAH1 and other members of the highly conserved SINA/SIAH subfamily of RING-type E3 ligases are composed of several domains: An N-terminal RING domain that interacts with an E2, two zinc-finger subdomains and a C-terminal substrate-binding domain (SBD). Here, we present the first crystal structure of the human SIAH1 RING domain, together with an adjacent zinc finger, revealing the formation of a tight RING dimer. The crystallised SIAH1 fragment is also dimeric in solution, and sequence conservation analysis and structural predictions indicate that RING dimerisation is an evolutionarily conserved feature of the SINA/SIAH E3 ubiquitin ligases. The SBD domain was previously identified as undergoing dimerisation within SINA/SIAH E3 ligases, and we propose that alternating RING:RING and SBD:SBD interactions organise SINA/SIAH proteins into higher-order assemblies. *In-vivo*, transient overexpression experiments performed in cultured human cells show that full-length human SIAH1 localises to elongated, filament-like cytoplasmic structures but shift to a diffuse distribution, when either RING or SBD is deleted or when RING dimerisation is disabled through a single point mutation. We hypothesise that this mechanism allows robust recruitment of multivalent targets, explaining the apparent preference of SINA/SIAH ligases for polymeric and aggregated protein substrates.

*Intervenant

Histone assembly mechanism coupled to DNA synthesis

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Résumé

Most cancers or cellular aging processes result not only from the alteration of gene sequences but also from the loss of epigenetic information that regulates gene expression. Essential epigenetic marks carried by histones may be lost during DNA replication or DNA repair, processes that require dissociation and reassembly of nucleosomes. Chromatin Assembly Factor 1 (CAF-1) is a three subunits complex, conserved in all eukaryotes, that orchestrates the assembly of histones H3-H4 coupled to DNA synthesis in the context of DNA replication and repair (1). This histone chaperone is thus particularly important for the maintenance of cell identity, but its action mechanism remains poorly understood. It has been established that its association with the DNA polymerase processivity factor, PCNA, is required for its functions during DNA replication, heterochromatin maintenance, and genome stability (2-3). A model for the histone deposition mechanism has been proposed from studies of a truncated S. cerevisiae CAF-1 complex (4), but we lack a framework to demonstrate its generality and how histone deposition is coupled to PCNA interaction.

We have undertaken structure function studies of the CAF-1 complex from yeast S. pombe. In particular, we investigate how this complex (composed of pcf1, pcf2 and pcf3) and its constitutive domains interact with different partners to deliver histones into DNA. We focused on CAF-1 interactions with DNA, histones H3-H4 and PCNA. We established that the ED domain of Pcf1 mediates histone binding and promotes conformational changes in CAF-1 (5). We are now exploring how additional interactions of histones H3-H4 with the two other subunits, Pcf2 and Pcf3 may trigger this conformational change. Regarding CAF-1 in full complex, we are studying the interplay between DNA and PCNA interactions with the aim of obtaining a high-resolution structure of this large complex. Here, we present the results we have obtained so far, through an integrative approach that combines biochemistry and structural biology methods, including NMR and SAXS, and bioinformatics modeling.

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- Pietrobon et al. 2014 10.1371/journal.pbio.1001968.
- Hardy et al. 2019; 10.1371/journal.pgen.1008441.
- Sauer et al. 2017; 10.7554/eLife.23474.
- Ouasti et al. 2024 ; 10.7554/eLife.91461.

I2BC Crystallography facility

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Résumé

X-ray crystallography is one of the most favored techniques for the determination of the atomic structure of proteins, nucleic acids and other molecules. The crystallography facility can provide service and scientific support to clients for their structural biology projects. Workflow for X-ray protein structure determination consists of crystal, diffraction pattern, electron density maps and protein model.
The platform is equipped with several nano-volume pipetting (including Lipidic cubic phase for membrane proteins) and visualization robots, which allow large-scale screening of crystallization conditions at two temperatures (4∘C and 19∘C). Additionally, the robots facilitate analysis and automated optimization of crystallization conditions for macromolecules. Services provided by the platform include self-service access to crystallization and visualization robots, provision of ready-to-use crystallization kits, and project delivery encompassing structure determination and analysis, including AlphaFold service and model analysis.

The crystallography platform benefits of the proximity of the Synchrotron Soleil and collaborate with the PROXIMA 2 beamline for the development of *in situ* collected data.

It is IBiSA labeled (Biology Health and Agronomy Infrastructures) and has the support of the "French Infrastructure for Integrated Structural Biology" (FRISBI). It is open to the entire scientific community including academia and industry.

The platform belongs to éciprocs (**Ré**seau des **C**hercheurs et **I**ngénieurs **Pro**fessionnels de la **C**ristallographie **S**tructurale).
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La protéine NS4 du norovirus humain s'auto-assemble en différents multimères de pontage membranaire

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Résumé

Les norovirus humains (HuNoV) sont des virus à ARN (+) extrêmement infectieux, responsables des épidémies de gastro-entérites aigües traversant les frontières et touchant toutes les classes d'âge. Chaque année, on estime à environ 700 millions le nombre de cas avec plus de 200 000 décès dans le monde et pourtant il n'existe à ce jour ni vaccin ni traitement antiviral spécifique. La réplication des huNoV nécessite la traduction de l'ARN génomique viral en une longue polyprotéine de réplication ORF1 (191kDa). L'ORF1 est clivée de manière co- et post-traductionnelle par une protéase (NS6) codée par le virus générant des intermédiaires de maturation et six protéines finales non structurales de NS1-2 à NS7. Un point crucial dans le cycle de vie des HuNoV est le remodelage de la membrane de l'hôte pour former un compartiment de réplication viral qui fournit un microenvironnement favorable à la réplication de l'ARN viral. Doerflinger et al. ont démontré que les protéines NS1-2, NS3 et NS4 interviennent dans les réarrangements membranaires intracellulaires associés à la réplication virale. Parmi elles, NS4 est la protéine clé du remodelage membranaire induits par les HuNoV, capable à elle seule de reproduire les déformations membranaires du réticulum endoplasmique observées lors d'une infection virale. Ce poster présentera des résultats récemment publiés dans JBC1 sur la façon dont la protéine NS4 s'autoassemble pour former différents types d'oligomères suite à son interaction avec des membranes artificielles (liposomes). Les résultats obtenus par cryo-microscopie électronique avec un microscope LaB6 120 kV ont depuis été étendus grâce à un Glacios FEG 200 kV et montrent des pontages des liposomes par NS4. La caractérisation de ces objets par cryo-tomographie électronique est en cours. 1. Royet, A., Ruedas, R., Gargowitsch, L., Gervais, V., Habersetzer, J., Pieri, L., Ouldali, M., Paternostre, M., Hofmann, I., Tubiana, T., et al. (2024). Nonstructural protein 4 of human norovirus self-assembles into various membrane-bridging multimers. J Biol Chem *300*, 107724. https://doi.org/10.1016/j.jbc.2024.107724.

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Moss BRCA2 interacts with recombinases in DNA repair

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Résumé

In vertebrates, the BRCA2 protein is essential for somatic and meiotic homologous recombination (HR) due to its interactions with RAD51 and DMC1 strand exchange proteins (recombinases). These interactions are mediated by conserved A- and P-motifs. A-motifs, present in the BRC repeats in the central region of BRCA2, compete with the A-motif of the linker region of RAD51 that is responsible for its self-oligomerization (Pellegrini et al., Nature 2002). Thus, BRC repeats form a binding scaffold for monomeric recombinases. P-motifs, present in the central and C-terminal regions of BRCA2, bind to oligomeric recombinases, and stabilize the RAD51 and DMC1 nucleoprotein filaments (Appleby et al., Nat Commun 2023; Miron et al., NAR 2024).
Human BRCA2 is a very large, 3418-aa, protein with a single folded DNA-binding domain. Its biochemical characterization is therefore challenging. The team of Dr Rajeev Kumar has recently identified a non-canonical BRCA2 protein in the model plant Physcomitrella patens. This protein, PpBRCA2, differs from the human protein as it exhibits only 391 amino acids and lacks a folded DNA-binding domain. However, it has 4 A-motifs and 2 P-motifs. *Physcomitrella patens* also expresses RAD51 (PpRAD51-1 and -2) and DMC1 (PpDMC1), which have approximatively 70% and 60% identity with human recombinases, respectively. The presence of these motifs and recombinases suggests that similar HR mechanisms exist between the two species. To support this hypothesis, I confirmed using Nuclear Magnetic Resonance that PpBRCA2 is totally disordered. I observed that it forms transient a-helices in its C-terminal region, containing the A-motifs. I further characterized the interactions between PpBRCA2 and DNA, as well as A- and P-motifs and recombinases, using BioLayer Interferometry. I found that its N-terminal region is responsible for ssDNA binding as well as RAD51-2 binding, whereas its C-terminal region composed of BRC repeats, only bind to RAD51-2.

We then used our plant model to identify new BRCA2 partners in HR. The team of Dr Rajeev Kumar identified potential partners by co-immunoprecipitation and mass spectrometry, and I analyzed their results using AF2-Multimer. Thus, I predicted binding of PpBRCA2 with both homologs of known partners of human BRCA2 (RAD51-1/2, RPA1) and new partner candidates. One of these new candidates is RADA, a protein homologous to bacterial

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RadA that is a DnaB-type helicase promoting bidirectional D-loop extension in HR (Marie et al., Nat Commun 2017). I am now experimentally verifying these interactions and performing structural studies of the PpBRCA2 complexes. I also designed first PpBRCA2 point mutations based on my AF2-Multimer analyses, to be tested *in vivo* by the team of Dr Rajeev Kumar.

DMC1 and RAD51 bind FxxA and FxPP motifs of BRCA2 via two separate interfaces
Simona Miron, Pierre Legrand, Sari E. van Rossum-Fikkert, Dejan Ristic, Atifa Badar, Roland Kanaar, Sophie Zinn-Justin, Alex N. Zelensky. *Nucleic Acids Research 2024*; doi: https://doi.org/10.1093/nar/gkae452.

Noncanonical ubiquitylation: modification of ADP-ribose and nucleic acids by DELTEX-family ubiquituin E3 ligases

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Résumé

In this poster, I cover some recent insights into noncanonical ubiquitylation, i.e. ubiquitylation of protein amino-acid residues other than lysine, as well as ubiquitylation of molecules or moieties other than protein amino-acid residues. In particular, I will focus on our work on DELTEX-family ubiquitin E3 ligases, which are capable of efficiently ubiquitylating an ADP-ribose moiety in vitro. The modified ADP-ribose can be present as a free molecule, as part of NAD+, or as part of a post-translationally ADP-ribosylated protein or post-transcriptionally ADP-ribosylated nucleic acid. Furthermore, I will share recent results concerning direct ubiquitylation of single-stranded nucleic acids by select DELTEX E3 ligases.

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Protein Cage Nanocompartments: Integrative Structural Biology Studies and Biotechnological Approaches for Future Therapeutic Applications

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Résumé

Protein cages, or nano-compartments, are common in nature, creating hollow spaces that serve as biomolecular containers for other molecules such as nucleic acids and proteins. Living organisms use protein cages to concentrate and compartmentalize enzymes and substrates, thereby accelerating catalysis and controlling the diffusion of toxic or volatile intermediates. In this work, we focus on the Encapsulin family-microbial protein compartments that form icosahedral shells specialized for cargo loading. We will present various integrative research axes pursued by our team, including computational studies on protein cages, such as molecular dynamics simulations and drug discovery targeting both the protein cages and related proteases. Additionally, we will share biochemical strategies for the production and purification of these challenging protein systems, as well as our pioneering hNH fast spinning solid-state NMR experiments on encapsulins.
Furthermore, we will discuss biotechnological approaches, such as glyco-conjugation strategies for protein cages and the development of encapsulins with micro-RNA loading capabilities

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Structural analysis of TopBP1, a scaffolding protein recruited to DNA damage sites to trigger repair.

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Résumé

Biomolecular condensates are micrometer-sized compartments that lack surrounding membranes but function to concentrate proteins and nucleic acids. Recent studies have demonstrated that topoisomerase IIβ-binding protein (TopBP1) assembles intra-nuclear condensates, driving the activation of ataxia telangiectasia-mutated and Rad3-related (ATR) checkpoint signaling pathway in response to DNA replication impediments. However, the precise structural mechanisms of how TopBP1 forms biomolecular condensates remain poorly understood.
Human TopBP1 is a prototype of "scaffold" protein composed of nine well-folded BRCA1 C-terminal (BRCT) domains serving as a phosphopeptide-binding modules, and an ATR activation domain (AAD) (Yu et al., Science 2003). Importantly, the AAD is intrinsically disordered and it is essential and sufficient for the ATR activation (Zhou ZW et al. PLoS Genet. 2013). A point mutation (W1145R) within the AAD abolishes TopBP1 condensate formation and ATR activation. Moreover, condensation favors phosphorylation of S1138 in the AAD, and phosphomimetic mutation S1138D increases the number of TopBP1 condensates (Frattani et al. Mol Cell. 2021). Despite the important role of the AAD in ATR activation, the AAD alone is insufficient for TopBP1 condensation. Recent studies have implicated the BRCT4/5 domains of TopBP1 in its condensation ability.

Inspired by this, we hypothesized that the AAD interacts with the BRCT4/5 of TopBP1, thereby contributing to the transient interactions responsible for condensation. Our experimental data revealed that a short region (1132-1155) of AAD, containing W1145 and S1138, interacts with BRCT4/5 with micromolar affinity by biolayer interferometry (BLI). Phosphorylation of S1138 enhanced binding affinity, whereas the W1145R mutation significantly reduced it. These results suggest that the AAD can directly interact with BRCT4/5 following phosphorylation at S1138.

AAD contains three predicted α-helices, which may be structurally important as they may correspond to binding sites. Nuclear magnetic resonance (NMR) spectroscopy, specifically

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13C, 15N chemical shift and 1H-15N heteronuclear nuclear Overhauser effect (NOE) analyses, confirmed that the AAD contains three partially populated α-helices. We further tested the direct interaction between full-length AAD and BRCT4/5 and observed a clear interaction involving the α-helices of the AAD, even in absence of the phosphorylation.
Our characterization of the AAD-BRCT4/5 interaction provides insight into a potential regulatory mechanism by which TopBP1 forms condensates during ATR activation. We first want to know the impact of the phosphorylation by ATR for AAD-BRCT4/5 interaction. We aim to further investigate this interaction by X-ray crystallography and assess the impact of the mutation of key interface residues in cells. Additionally, we plan to explore how TopBP1-TopBP1 interactions are regulated throughout the cell cycle.

Regulation of the leukemia-related BCR protein at the membrane interface

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Résumé

The BCR protein (Breakpoint cluster region) is mainly known as part of the BCR-ABL1 chimera, an oncogene causing leukemia. Outside of its oncogenic role in lymphocytes, BCR function is still poorly understood. Although BCR has been shown to regulate Rho GTPases-a family of small GTPases that control cytoskeleton dynamics-many questions remain about its cellular substrates and its precise physiological role in lymphocytes. Rho GTPases act as molecular switches that cycle between an inactive, GDP-bound state in the cytosol and an active, GTP-bound state at the membrane. Their activation are tightly regulated by membrane association, as well as by regulators that also interact with membranes to modulate their activity.
Based on this, our first question was "*how BCR activity is modulated by its interaction with cellular membranes ?*". Using a flotation assay, we demonstrated that a truncated BCR construct containing the membrane-interacting PH domain binds strongly to negatively charged lipids. In contrast, the full-length BCR adopts an auto-inhibited conformation in solution, resulting in low membrane recruitment. Our current objective is to determine the inactive, soluble conformation of BCR using cryoEM to validate our result of flotation assay and clarify the mechanism by which the membrane recruitment is inhibited. We obtained a low-resolution envelop representing approximately half of the BCR size, which enabled us to fit the portion of BCR predicted by AlphaFold to be properly folded. To achieve higher resolution, we have optimized the GraFix method, and preliminary results are presented here.

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