BSI 2025

4TH INTEGRATIVE STRUCTURAL BIOLOGY MEETING

15-19 DECEMBER 2025 • BORDEAUX, FRANCE

BOOK OF ABSTRACTS

Contact:
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FOREWORD:

Dear Colleagues and Participants,

It is our great pleasure to welcome you to the 2025 edition of the Integrative Structural Biology meeting (BSI4). Following the success of the inaugural event in Toulouse in 2019, the second edition in Paris-Saclay in 2021, the third edition in Marseille in 2023, this forth meeting brings together 200 attendees at the Domaine du Haut Carré, in the vibrant and historic campus of Université Bordeaux in Talence, a setting we hope you will fully appreciate.

In just a few years, the BSI meetings have accompanied remarkable advances in both structural and cellular biology. Rapid developments in cryo-electron microscopy and super-resolution optical imaging have overcome previous technological limits, enabling unprecedented views of proteins, organelles, cells, and tissues. These rapidly evolving tools continue to reshape our understanding of biological mechanisms across multiple disciplines - including virology, microbiology, cancer research, neurobiology, cellular biology, and more - all reflected across the ten scientific sessions planned for BSI4. This meeting aims to highlight and encourage the growing collaboration among structural biologists, cell biologists, biochemists, and biophysicists, whose complementary approaches are increasingly essential.

BSI4 offers a unique environment to strengthen scientific connections across these research communities and to provide a broad overview of current advances addressing shared biological challenges from different technical angles. This event is made possible through the collaboration with the Societé Française de Biophysique (SFB) and the Association Française de Cristallographie (AFC), together with the help of the Scientific Committee, representing the diversity and strength of our national expertise. We have also placed particular emphasis on encouraging participation from early-career researchers and PhD students, while promoting diversity, equality, and inclusion.

The program includes two conferences Grand Public, three Keynote lectures, and many invited and selected talks, poster sessions and awards recognizing outstanding contributions. These scientific activities will be complemented by opportunities to meet and interact — including shared lunches, a welcome cocktail, and a gala dinner. On Wednesday evening, we will gather at the Cité du Vin in Bordeaux, for an exceptional dinner in a remarkable setting that invites conversation, networking, and celebration.

We are confident that BSI4 will provide an inspiring forum for discussion, collaboration, and discovery across the fields of structural biology, biophysics, and cellular biology. We look forward to your participation and to sharing an exciting and enriching meeting with you here in Talence and Bordeaux.

Antoine Loquet, Birgit Habenstein and Brice Kauffmann

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PROGRAMME

Monday 15 December

11h00-13h00 : Welcome participants

13h00-13h45 : Introduction, Organizing Committee, AFC and SFB President

13h45-16h00 : Session 1

13h45-14h15: Helene Malet : Structural and functional characterization of Bunyavirus replication and transcription

14h15-14h35: Ons Dakhlaoui : Structural basis and regulation of Cap-4 RNA binding to eIF4E1 in Trypanosoma cruzi revealed by an integrative structural biology approach.

14h35-14h55: Cyril Charlier : Mechanistic details of a PETase at high-temperature

14h55-15h15: Robert Bosman : Catalytic dynamics in an antibiotic resistance enzyme observed by time-resolved crystallography.

15h15-15h35: Vincent Chaptal : Motion Pictures. Visualizing protein intimate movements through inter-particle variability of cryo-EM data.

15h35-17h00: Coffee break

17h00-18h20: Session 2

17h00-17h30: Giorgio Schiro : Integrated structural approach tracks B12 photoreceptor activation in real time

17h30-17h50: Florence Tama : tomistic Biomolecular Dynamics from High-Speed Atomic Microscopy data

17h50-18h10: Antoine Royant : Protein dynamics probed at room temperature by combined time-resolved crystallography and spectroscopy

18h10-18h20: Gianluca Cioci : Structural Investigations of CAZymes for the Synthesis of Biopolymers

18h20-19h20: Conference Grand Public: Sylvain Delzon : Responses of forests and farming systems to global change

19h20-22h: Welcome cocktail

Tuesday 16 December

9h00-10h30 : Session 3

9h00-9h30: Adeline Goulet : Exploring bacteriophage structures with a focus on their host-binding machineries

9h30-9h50: Pierre Dorlet : An original periplasmic system for copper resistance in bacteria

9h50-10h10: Eleni Litsardaki : Histone assembly mechanism coupled to DNA synthesis

10h10-10h30: Birgit Strodel : A holistic approach to enzymatic plastic degradation: from machine learning and simulations to wet lab and back.

10h30-11h00: Coffee break

11h00-12h30: Session 4

11h00-11h30: Massimiliano Bonomi : Structural and dynamic biology with integrative approaches

11h30-11h50: Elsa Garcin : Engineering SIRPα conformational plasticity to reveal a cryptic pocket suitable for structure-based drug design

11h50-12h10: Julien Rizet : Is Mycobacterial InhA a Suitable Target for Rational Drug Design?

12h10-12h30: Mathieu Chavent : Modelling host-pathogen interactions using multiscale MD simulations: the Mycobacterium tuberculosis case

12h30-15h00: Lunch + Poster session

15h00-15h50: Keynote 1 – Sebastian Hiller : A structural journey through molecular life and death

15h50-16h30: Industrials

16h30-17h00 : Coffee break

17h00-18h30 : Session 5

17h00-17h30: Ariane Briegel : Using a large volume cryo-electron tomography pipeline to study microbial interactions with their environment

17h30-17h50: Shrankhla Bawaria : Structural and functional studies of CHIKUNGUNYA NSP1-4 with its potent inhibitors.

17h50-18h10: Juan Reguena : Decoding how Chikungunya virus non structural proteins assemble in evolving complexes for seting a sequential program of virus infection

18h10-18h30: Célia Caillet-Saguy : Sars-cov-2 Envelope protein–host ZO-1 interaction: from virulence mechanisms to targeted inhibitor discovery

Wednesday 17 December

9h00-10h30 : Session 6

9h00-9h30: Rebekka Wild : Mechanistic insight into the step-by-step biosynthesis of glycosaminoglycans

9h30-9h50: Suliane Quilleré : Moss BRCA2 interacts with recombinases and ssDNA in DNA repair

9h50-10h10: Julien Karazi : The role of the WRN exonuclease/helicase in coordinating DNA repair and replication pathways, and their connection to premature aging syndromes

10h10-10h30: Pierre Maisonneuve : Novel Regulatory Mechanisms of the Pseudokinase KSR in the RAS-ERK Pathway

10h30-11h00: Coffee break

11h00-12h20: Session Infrastructure

12h20-15h00: Lunch + Poster session

15h00-18h00: Free time – travel to Cité du Vin

18h00-19h00: Conference Grand Public: Daniel Choquet : Nanoscale synapse organization and function

19h00-20h00 : Visit of the Musem

20h00-23h00 : Gala Diner

Thursday 18 December

9h00-10h30 : Session 7

9h00-9h30: Lionel Mourey : Quaternary structure and DNA recognition of a toxin-antitoxin-chaperone system of Mycobacterium tuberculosis

9h30-9h50: Andreas Zoumpoulakis : Unique structural features of the human Kir2.1 channel in two different conformational states unveiled by cryo-electron microscopy.

9h50-10h10: Paramita Chaudhuri : Condensation of an intrinsically disordered translation factor eIF4B: from in vitro to in-cell

10h10-11h00: Coffee break

11h00-11h50 Keynote 2 – Malene Jensen : Elucidating the interaction mechanisms of intrinsically disordered proteins at atomic resolution

11h50-15h00: Lunch + Poster session

15h00-15h50: Session 8

15h00-15h30: Marion Mathelie-Guinlet : N-terminal capping tunes fibrillation and cytotoxicity of Staphylococcus aureus PSMα3

15h30-15h50: Pascale Marchot : Molecular bases for functional disruption of synapse by autoantibodies in limbic encephalitis.

15h50-16h10: Bertrand Raynal : Role of monomers, Dimers, and Oligomers of Pyroglutamate-Modified α-Synuclein Fragments in fibril assembly.

16h10-16h30: Nadia El Mammeri : Understanding Tau's Chemical code: microtubules, lipid membranes, and phosphorylation

16h30-17h30: Coffee break

17h30-18h30 : Session 9

17h30-18h00: Lauriane Lecoq : Bridging Solution and Solid-State NMR to Elucidate Hepatitis D. Virus Protein-RNA Interactions

18h00-18h20: Leo Chillard : Balancing activation and inhibition of immune receptors: AlphaFold-guided structural analysis of the paired NLRs RGA4 and RGA5

18h20-18h40: Romain La Rocca : Structure of a photosystem II-FCPII supercomplex from a haptophyte reveals a distinct antenna organization.

Friday 19 December

9h00-10h20: Session 10

9h00-9h40: Prix SFB – Jody Pacalon

9H40-10h00: Erick Dufourc : Quantitative lipid analysis of brain, myelin-enriched and corpus callosum fractions in mice, by multinuclear NMR and mass spectrometry

10h00-10h20: Maria Davila Miliani : Time-resolved cryo-EM to visualize biomolecules in action

10h20-10h40: Coffee break

10h40-11h30: Keynote 3 – Petya Krasteva : Mechanisms of activation and diversification of the bacterial cellulose synthase

11h30-12h00: Concluding remark, remise prix poster, talk, prix Cancéropole.

Thanks to our supports:

AFC Sponsors:

ORAL PRESENTATIONS

(In the order of the program)

#10899

Structural and functional characterization of Bunyavirus replication and transcription

Helene MALET (IBS)

Topics

N/A

Communication type

Poster

Abstract

Bunyaviricetes is an order of segmented negative-stranded RNA viruses comprising over 500 species in fifteen families. Widespread globally, they threaten human and animal health, with several bunyaviruses on the WHO priority list for urgent countermeasure development. Among them, Hantaan virus (HTNV, Hantaviridae) causes hemorrhagic fever with renal syndrome, and La Crosse virus (LACV, Peribunyaviridae) causes encephalitis in newborns. Studying these viruses is essential to understand their function and identify antiviral targets. We focus on two key steps of their cycle: (i) genome replication and (ii) transcription via “cap snatching,” producing mRNAs for viral proteins. Both are catalyzed by the RNA-dependent RNA polymerase (L), which binds the 3′ and 5′ ends of viral RNA segments. The polymerase acts on the nucleocapsid (NC), where RNA is encapsidated by nucleoproteins, forming ribonucleoprotein particles (RNPs) that are the active units of replication and transcription. We present new high-resolution cryo-EM structures of full-length HTNV and LACV polymerases, revealing their complex organization and conformational rearrangements during activity. These structures, together with biochemical data and the high-resolution HTNV NC model, provide detailed snapshots of polymerases in action and lay the groundwork for structural and functional studies of viral replication within RNPs.

#10374

Structural basis and regulation of Cap-4 RNA binding to eIF4E1 in Trypanosoma cruzi revealed by an integrative structural biology approach

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Topics

RNA/DNA

Communication type

Oral

Abstract

Trypanosoma cruzi, the causative agent of Chagas disease (Kaufer et al., 2017), represents a major public health challenge, with millions of cases worldwide, increasing incidence, and ongoing geographic expansion (Niborski et al., 2021). Current chemotherapeutic options are limited by poor selectivity and severe side effects (Sueth-Santiago et al., 2017), underscoring the need for new therapeutic strategies. A key peculiarity of T. cruzi biology is its reliance on unique mechanisms of gene regulation. Unlike higher eukaryotes, trypanosomes lack canonical transcriptional control and instead rely on polycistronic transcription coupled with post-transcriptional regulation, particularly translation initiation. Central to this process is the hypermethylated cap-4 RNA modification, specifically recognized by the translation initiation factor eIF4E1. This protein contains two lineage-specific sequence insertions predicted to be intrinsically disordered, raising the question of their potential regulatory role. Yet, the molecular basis of cap-4 recognition and its regulation by accessory factors—such as eIF3A (activator) and 4E-IP1 (inhibitor)—remains poorly defined (Falk et al., 2021).

Here, we present an integrative structural biology study that dissects the interactions of T. cruzi eIF4E1 with its RNA ligand and protein partners. Quantitative binding analyses were performed using microscale thermophoresis (MST), isothermal titration calorimetry (ITC), and surface plasmon resonance (SPR). To resolve molecular interfaces, we combined NMR spectroscopy—exploiting the tractable size of several partners—with hydrogen–deuterium exchange mass spectrometry (HDX-MS) when NMR was not feasible. Small-angle X-ray scattering (SAXS) experiments complemented these data and, together with AlphaFold predictions, enabled structural modeling of complexes.

Our results demonstrate that eIF4E1 binds cap-4 RNA and the inhibitor 4E-IP1 simultaneously, with conserved recognition features resembling those in higher eukaryotes. Functional assays reveal that 4E-IP1 does not displace cap-4 RNA but instead inhibits recruitment of downstream initiation factors such as eIF3A, suggesting a competitive regulatory mechanism. Biochemical competition assays confirmed that 4E-IP1 and eIF3A share overlapping binding regions. NMR titrations further showed that the eIF4E1 disordered insertions do not contribute to cap-4 or 4E-IP1 recognition, but instead mediate interaction with eIF3A. This represents, to our knowledge, the first evidence of a functional role for these disordered segments in modulating translation factor binding. Consistently, SAXS analyses revealed that the disordered eIF3A fragment (residues 665–762) undergoes structural ordering upon complex formation with eIF4E1.

Together, our data define the molecular determinants of eIF4E1–cap-4 recognition and its regulation by inhibitory and activating partners. By revealing both conserved and parasite-specific features, this work advances our understanding of translation initiation in T. cruzi and highlights eIF4E1 regulatory interfaces as promising targets for selective therapeutic intervention.

#10261

Mechanistic details of a PETase at high-temperature

M Cyril CHARLIER (Toulouse Biotechnology Institute (TBI, Toulouse))
M Cyril CHARLIER (Toulouse Biotechnology Institute (TBI, Toulouse))

Topics

Methods

Communication type

Oral

Abstract

Polyethylene terephthalate (PET) is one of the most widely used plastics worldwide, but its persistence in the environment has raised major concerns about plastic pollution. Enzymatic recycling has emerged as a promising route toward sustainable plastic management, with PET hydrolases (PETases) showing the ability to depolymerize PET into its constituent monomers for reuse in the production of new, high-quality plastics. Among these enzymes, LCC-ICCG, a bioengineered variant of the leaf-branch compost cutinase (LCC), represents a powerful industrial candidate due to its superior thermal stability and catalytic performance. However, despite important advances in rational design and machine-learning-driven optimization, the mechanistic details governing PETase function remain insufficiently understood, particularly at the molecular level and under conditions that mimic industrial settings. In this presentation, we will highlight how advanced nuclear magnetic resonance (NMR) spectroscopy provides new insights into the catalytic mechanism, structural dynamics, and thermal resilience of LCC-ICCG, paving the way toward improved strategies for PET enzymatic recycling.

We first explore the role of histidine residues in the catalytic mechanism of PET hydrolysis. Belonging to the carboxylic-ester hydrolase family, PETases feature a canonical Ser-His-Asp catalytic triad. Using NMR, we determined the tautomeric states and pKa values of the six histidines in LCC-ICCG, revealing that five residues exhibit unexpectedly low pKa values below 4.0. Remarkably, the catalytic histidine (H242) displayed a pKa value that decreased from 4.9 at 30 °C to 4.7 at 50 °C. This subtle shift correlates with the enzyme's hydrolytic activity in solution but diverges significantly when the substrate is crystalline PET, underscoring the importance of investigating enzymatic function at the liquid–solid interface. These findings provide a more refined picture of how protonation dynamics modulate PETase catalysis and suggest directions for further mechanistic exploration.

In parallel, we addressed a major bottleneck in NMR-based structural studies of PETases: the time-consuming acquisition and analysis of triple-resonance spectra required for resonance assignment. By recording spectra at elevated temperatures, which also reflect realistic operational conditions, we significantly improved the signal-to-noise ratio and spectral quality. Using an inactive LCC-ICCG variant, we demonstrated that backbone assignment can be completed using a minimal set of spectra acquired at 50–60 °C, reducing the timescale for assignment to less than two weeks. This achievement places NMR on a comparable footing with crystallography in terms of speed, while offering unique opportunities to probe enzyme dynamics under working conditions.

In conclusion, by integrating mechanistic studies, methodological advances, and comprehensive structural assignments, we demonstrate how NMR can uniquely illuminate the structure–function relationships of PETases. These advances not only enhance our fundamental understanding of enzyme catalysis at the liquid–solid interface but also contribute directly to the development of scalable biotechnological solutions for plastic recycling.

#10368

Catalytic dynamics in an antibiotic resistance enzyme observed by time-resolved crystallography.

Robert BOSMAN (Universitätsklinikum Hamburg-Eppendorf)
David VON STETTEN (European Molecular Biology Laboratory (EMBL))
Eike SCHULZ, C (University Medical Center Hamburg-Eppendorf (UKE))

Topics

Methods

Communication type

Oral

Abstract

Antibiotic resistance is often mediated by antibiotic modifying enzymes (AMEs). Aminoglycoside Acetyltransferases (AAC) are members of the (GCN5)-related N-acetyltransferases (GNAT) family which mediate non-protein Acetyl-CoA dependent acetylation. Different AAC's are responsible for aminoglycoside inactivation by acetylating a primary amine at a specific position on aminoglycoside sugar rings[1]. This prevents the aminoglycoside binding to the ribosome A-site, which would disrupt protein translation. Interestingly, AAC-(6')-il is a dimeric enzyme that displays distinct temperature-dependent cooperative responses to either binding the acetyl-CoA, or aminoglycoside antibiotic substrates[2]. Moreover, like many AAC's, it is promiscuous to 4,5- and 4,6-linked aminoglycoside antibiotics, however unlike others, in AAC-(6')-il the aminoglycoside pocket is located between dimer subunits likely changing the interactions between dimers with different substrates[3].

Despite this, there is currently neither a structure for the ternary complex nor any for catalytic intermediates. We aim to understand the structural changes underpinning the allosteric communication and how it is related to acetylation. To this end we utilize our method developments in multi-temperature, time-resolved serial crystallography, and automated cryo-trapping via the spitrobot crystal plunger on the AAC-(6')-il:AcCoA complex[4,5,6].

These methodologies permit us to measure crystal structures in out-of-equilibrium conditions and determine the structure of meta-stable states. As such, we report the first aminoglycoside bound structure to AAC-(6')-il, as well as an ES complex alongside catalytic intermediates. We demonstrate that AAC adopts distinct conformations dependent on binding either its 4,5- and 4,6- linked substrates. Finally, we highlight the potential catalytic role of a previously unobserved water molecule appearing in the active-site upon aminoglycoside binding.

1. Krause, K. M., Serio, A. W., Kane, T. R. & Connolly, L. E. Aminoglycosides: An Overview. Cold Spring Harb. Perspect. Med. 6, a027029 (2016).

2. Freiburger, L. et al. Substrate-dependent switching of the allosteric binding mechanism of a dimeric enzyme. Nat. Chem. Biol. 10, 937–942 (2014).

3. Burk, D. L., Ghuman, N., Wybenga-Groot, L. E. & Berghuis, A. M. X-ray structure of the AAC(6′)-Ii antibiotic resistance enzyme at 1.8 Å resolution; examination of oligomeric arrangements in GNAT superfamily members. Protein Sci. 12, 426–437 (2003).

4. Mehrabi, P. et al. Liquid application method for time-resolved analyses by serial synchrotron crystallography. Nat. Methods 16, 979–982 (2019).

5. Mehrabi, P. et al. Millisecond cryo-trapping by the spitrobot crystal plunger simplifies time-resolved crystallography. Nat. Commun. 14, 2365 (2023).

6. Schulz, E. C. et al. Probing the modulation of enzyme kinetics by multi-temperature, time-resolved serial crystallography. Na Commun. 16, 6553 (2025).

#10244

MOTION PICTURES. VISUALIZING PROTEIN INTIMATE MOVEMENTS THROUGH INTER-PARTICLE VARIABILITY OF CRYO-EM DATA

Vincent CHAPTAL (MMSB UMR5086)

Topics

Methods

Communication type

Oral

Abstract

Macromolecules undergo complex movements to perform their function. These movements can be made of large conformational changes or small local rearrangements depending on the function at play or the space explored by the macromolecule at this moment.
One main interest of cryoEM is that it captures macromolecules “in the act” as particles are frozen while the macromolecule undergoes either large or small conformational changes. It is then possible to visualize the movement by studying how particles are different from one another.
We have visualized movements for the multidrug transporter BmrA revealing how ligands influence the dynamics of the transporter therefore creating asymmetry within a symmetric transporter1; we have visualized the influence of single mutations on the asparagine synthase overall vibration2; and we have visualized on the azole transporter Cdr1 the opening resulting from ATP hydrolysis and the piston-like mechanism following ATP hydrolysis (under review, Nat. Comm.). All these observations open the way to a new understanding of the functioning of these proteins and pave the way to new discoveries. These observations were possible through the development of variability refinement, a tool that is publicly available free to academics in the Phenix software suite.

Annexes

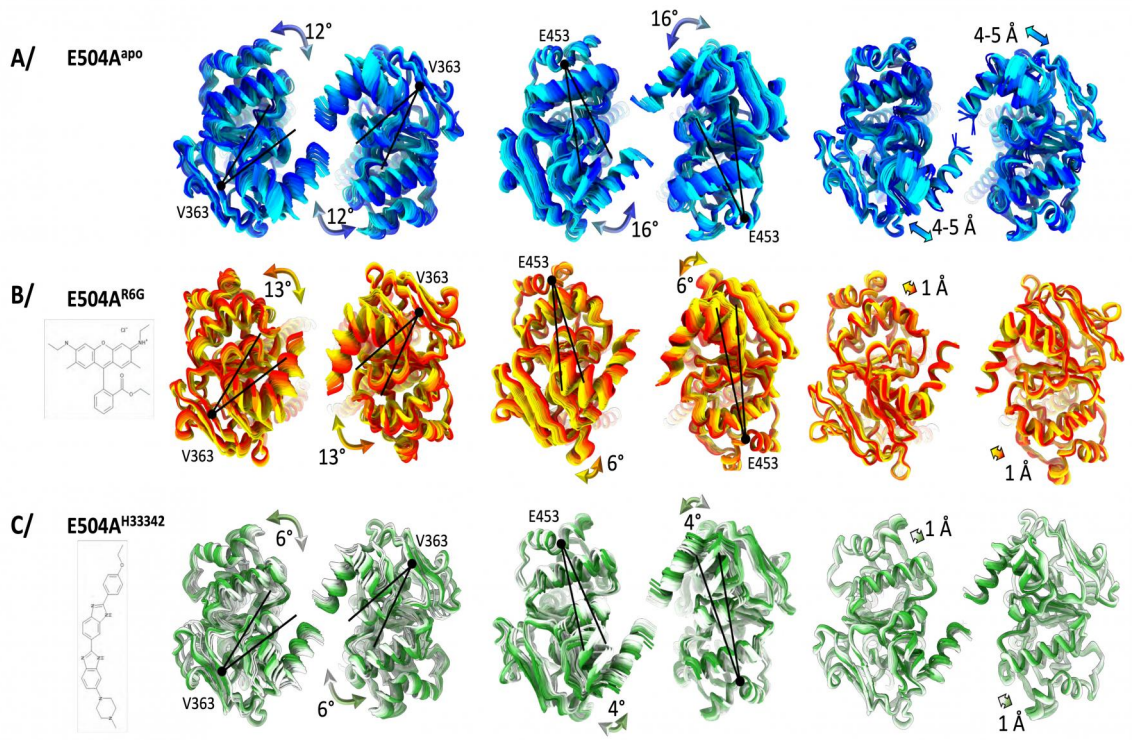

Figure 1: influence of ligands on the conformational space exploration by the ABC transporter BmrA

#10880

Integrated structural approach tracks B12 photoreceptor activation in real time

Giorgio SCHIRO (CNRS-IBS)

Topics

N/A

Communication type

Poster

Abstract

Photoreceptor proteins control several fundamental biological processes. A large photoreceptor subfamily uses vitamin B12 derivatives for light sensing, with a molecular mechanism largely unknown. Here I will report on a detailed spatio-temporal description of photoactivation in the prototypical tetrameric B12 photoreceptor CarH from nanoseconds to seconds using an integrative approach, combining time- and temperature-resolved structural and spectroscopic methods with quantum chemical calculations. High-resolution structural snapshots of key intermediates in the truncated B12 binding domain illustrate how photocleavage of a cobalt-carbon bond within the B12 chromophore triggers a series of structural changes that propagate throughout CarH, with the intermediate formation of a previously unknown adduct, subsequently cleaved thermally to allow release of the photocleaved group and tetramer dissociation. The biological relevance of our study is corroborated by time-resolved X-ray solution scattering data on full-length CarH in the presence of DNA. Our results offer an olistic spatio-temporal understanding of CarH photoactivation over nine orders of magnitude in time.

#10247

Atomistic Biomolecular Dynamics from High-Speed Atomic Microscopy data

Florence TAMA (RIKEN Center for Computational Science)

Topics

Methods

Communication type

Oral

Abstract

Understanding biomolecular function requires insights into not only static structures but also their dynamics. High-speed atomic force microscopy (HS-AFM) has become a powerful technique for directly visualizing biomolecular dynamics at the single-molecule level under near-physiological conditions. HS-AFM produces two-dimensional (2D) topographical images that capture conformational changes and functional motions in real time. However, because of its limited resolution, HS-AFM imaging alone cannot provide three-dimensional (3D) atomic models, making computational methods essential to bridge the gap between experimental data and atomistic interpretation.

We introduce a new flexible fitting method, NMFF-AFM, which combines computational modeling method of normal mode analysis with AFM image fitting to build 3D atomic models from 2D HS-AFM data. The approach utilizes low-frequency normal modes, known for capturing large-scale motions important for function, to deform a given protein structure and improve the fit to AFM images. To validate this method, we performed systematic tests with simulated AFM data from proteins with well-characterized conformational changes. This approach allowed us to carefully evaluate robustness in controlled settings. We also examined how rigid-body alignment procedures and potential misalignments, which are inevitable when working with experimental data, affect the results. Despite AFM's resolution limits, NMFF-AFM consistently captured the main conformational motions of biomolecules [1].

Building on these validations, we integrated NMFF-AFM into the widely used BioAFMviewer platform, establishing a streamlined workflow from raw HS-AFM movies to atomistic modeling and visualization. This user-friendly implementation enables direct analysis of experimental HS-AFM data and facilitates applications across various biomolecular systems. We demonstrate the versatility of the method through applications to experimental datasets, including a single protein domain, a multi-protein complex, and a megadalton-scale filament. In addition, application of NMFF-AFM to time-resolved HS-AFM sequences allowed reconstruction of atomistic molecular movies, directly linking experimental imaging to molecular-level dynamics [2].

In summary, NMFF-AFM provides a computationally efficient strategy to characterize atomistic biomolecular dynamics from HS-AFM data. Its integration into BioAFMviewer makes the approach widely accessible, paving the way for automated, large-scale analysis of HS-AFM imaging. The combination of experiment and computation offers new opportunities to test mechanistic hypotheses, guide experimental design, and advance our understanding of biomolecular dynamics.

[1] Wu X. et al. J. Phys Chem B, 2024

[2] Aymot R. et al. ACS Nano, 2025

Annexes

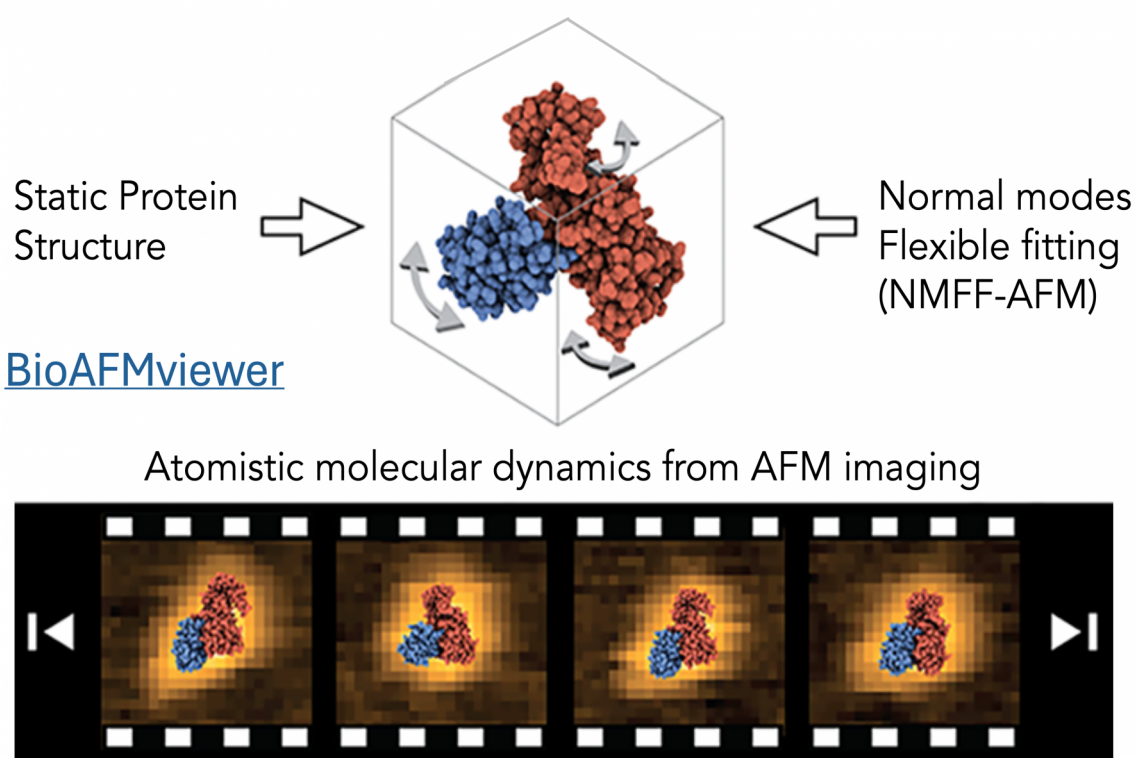

Figure 1: Integrative modeling of biomolecular dymanics from AFM data.

#10357

Protein dynamics probed at room temperature by combined time-resolved crystallography and spectroscopy

M Antoine ROYANT (Institut de Biologie Structurale)

Topics

Methods

Communication type

Oral

Abstract

Time-resolved X-ray crystallography is currently witnessing a rebirth at synchrotrons after X-ray free-electron lasers led the way [1]. The dynamics of light-activated proteins is now studied on the microsecond to minute timescale at room temperature. At the *ic*OS Lab at the ESRF, we have been developing spectroscopic methods to complement structural information with time-resolved spectroscopic data recorded on crystals on matching timescales. This approach helps identify potential artefacts resulting from the crystalline state or too high light fluences, and to sample the most relevant time points [2,3,4,5,6]. On beamline BM07-FIP2, we are developing different methods to study the dynamics of non-light-activated proteins. We use X-ray induced electrons to probe the oxidised-to-reduced transition of metal-containing proteins, using optical spectroscopy as a metrics of metal reduction [7]. Finally, we have set up an injector dispensing nanolitres of ligand solution onto crystals, in order to monitor enzymatic reactions on the second-to-minute timescale, shedding new insights on their mechanism.

[1] Caramello & Royant, *Acta Crystallogr.* D (2024)
[2] Aumonier *et al.*, *IUCrJ* (2020)
[3] Aumonier *et al.*, *IUCrJ* (2022)
[4] Engilberge *et al.*, *Acta Crystallogr.* D (2024)
[5] Maestre-Reyna *et al.*, *Sci. Adv.* (2025)
[6] Lamm *et al.*, *Sci. Adv.* (2025)
[7] Bolton *et al.*, *Proc. Ntl. Acad. Sci. USA* (2024)

#10370

Structural Investigations of CAZymes for the Synthesis of Biopolymers

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Topics

Other

Communication type

Oral

Abstract

Polymers are ubiquitous in modern life, spanning applications from everyday products to advanced technologies in food, health, and environmental sectors. Yet, less than 1% of the approximately 350 megatons produced annually are bio-based, with the vast majority still derived from fossil resources. The urgent need for sustainable and renewable alternatives has driven growing interest in bio-derived polymers that are recyclable, biocompatible, and potentially bioactive.[1]

Carbohydrate-active enzymes (CAZymes), capable of polymerization, glycosylation, phosphorylation, and related modifications, offer powerful biosynthetic routes to such materials.[2,3] However, engineering these enzymes requires a detailed understanding of their catalytic mechanisms, substrate interactions, and reaction dynamics—factors often obscured by their complex, multidomain, and dynamic architectures.

At TBI's Biocatalysis team, we are developing an integrative approach to characterize these enzymes using a suite of structural biology techniques, including cryo-electron microscopy, X-ray crystallography, small-angle X-ray scattering, and nuclear magnetic resonance, complemented by biochemical assays and molecular modeling. We will present recent insights into key enzyme families such as glucansucrases, glycoside phosphorylases, and glucan kinases, demonstrating how these complementary methods can elucidate enzyme conformational and reaction dynamics during catalysis. This integrated approach paves the way for rational enzyme engineering toward the sustainable synthesis of biopolymers and glycosylated compounds.

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Annexes

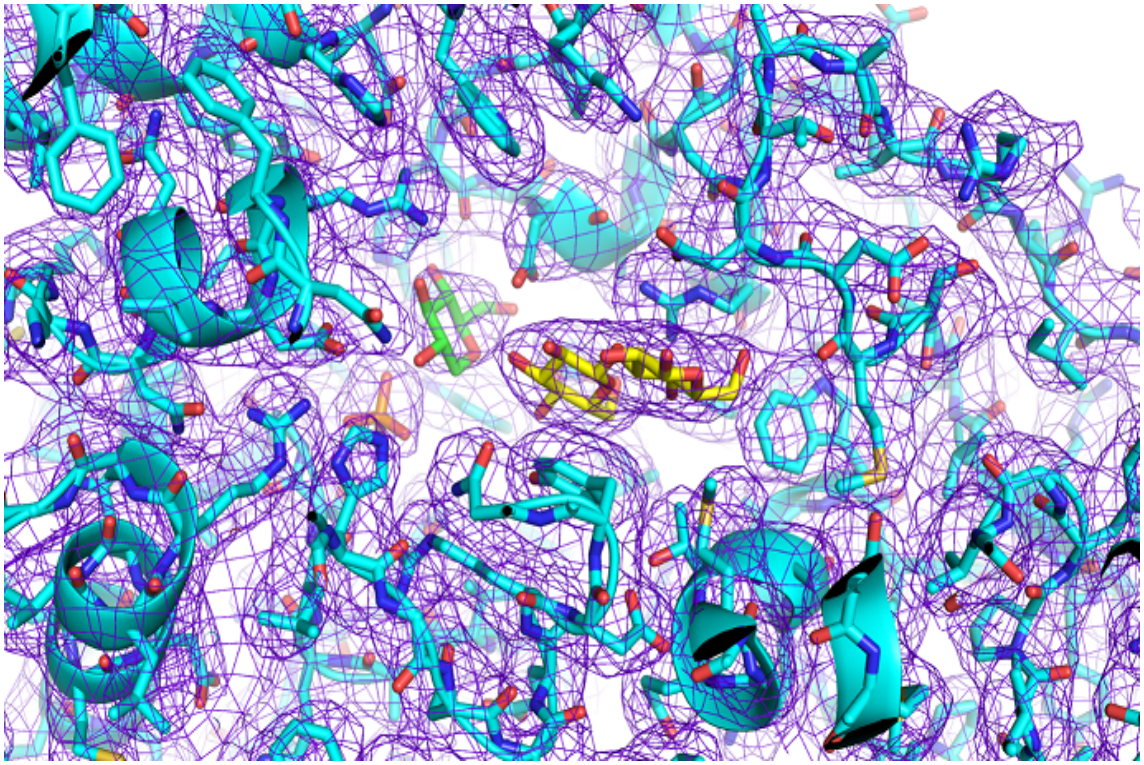

Figure 1: Catalytically-active glucan phosphorylase as obtained by cryo-EM

#10881

Exploring bacteriophage structures with a focus on their host-binding machineries

Adeline GOULET (CNRS)

Topics

N/A

Communication type

Poster

Abstract

Bacteriophages (phages), viruses that infect bacteria, are the most abundant and diverse biological entities on Earth. Their ability to recognize and kill a specific bacterium makes them either our enemies or our allies. Interactions between the host-binding machinery of phages, a multiprotein assembly located at their tail tip, and bacterial cell surface receptors trigger the infection cycle. Deciphering the structural and molecular basis of these interactions is therefore crucial for controlling phage-host interactions and preventing unwanted phage infections in industrial settings. Conversely, enhancing these interactions can be beneficial for phage-based diagnostic and therapeutic applications. In this presentation, I will discuss recent structures of various phage host-binding machineries, including those from phages infecting the winemaking lactic acid bacterium Oenococcus oeni and the human pathogen Mycobacterium abscessus. These structures, determined using experimental (cryo-electron microscopy) and/or computational (AlphaFold structure prediction) approaches, reveal the remarkable modularity and diversity of these LEGO® -like assemblies, which are built from a shared pool of structural and functional protein domains. They also illustrate how phages have evolved molecular adaptations to their host surfaces.

#10355

An original periplasmic system for copper resistance in bacteria

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Topics

Microbes

Communication type

Oral

Abstract

The antimicrobial properties of copper are commonly used in anthropogenic activities in agriculture and health. This has led to copper accumulation in the environment and contributed to the emergence of copper resistant microorganisms. Understanding bacterial copper homeostasis diversity is therefore highly relevant since it could provide valuable targets for novel antimicrobial treatments. For the past 15 years, genes encoding for small periplasmic proteins involved in copper resistance in bacteria have been reported but those proteins have been little characterized and sometimes not isolated nor studied.

We are interested in an operon from the photosynthetic purple bacterium Rubrivivax gelatinosus containing three genes encoding three periplasmic proteins (CopI, CopJ and CopH) allowing the bacterium to resist up to 1.2 mM of copper in the environment. Of importance, this bacterium does not possess genes encoding for copper oxidase or Cus system. Also, homologs of CopI are found in pathogenic bacteria such as Pseudomonas aeruginosa or Vibrio cholera. The periplasmic CopI protein[1] is a monodomain cupredoxin comprising several copper binding sites that we have characterized[2,3] by using different mutants targeting the putative copper binding modules and various spectroscopic techniques. Using NMR and crystallography, we have obtained a structure for the CopI protein which was unresolved till now. We have shown that CopI is able to bind a cuprous ion in its central histidine/methionine-rich region and to oxidize it thanks to its cupredoxin center[3]. Data regarding CopJ, which comprises a Cu cluster, and CopH, an intrinsically disordered protein rich in His and Met residues, will also be presented.

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#10328

Histone assembly mechanism coupled to DNA synthesis

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Topics

RNA/DNA

Communication type

Oral

Abstract

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 structural and functional studies of the CAF-1 complex from yeast S. pombe, using an integrative approach combining functional studies, biochemistry and structural biology methods, including NMR spectroscopy and SAXS, and bioinformatics modeling. In particular, we have investigated how this complex (composed of Pcf1, Pcf2 and Pcf3) and its individual domains interact with different partners to deliver histones onto DNA. We focused on CAF-1 interactions with DNA, histones H3-H4 and PCNA. We show that the ED domain of Pcf1 mediates histone binding and promotes conformational changes in CAF-1 [5]. We then explored how additional interactions of histones H3-H4 with the two other subunits, Pcf2 and Pcf3 may trigger this conformational change. We also investigated the interactions of the full CAF-1 complex with both DNA and PCNA, and here we present results revealing new binding interfaces and conformational adaptations within CAF-1 associated with its role in histone assembly.

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#10132

A holistic approach to enzymatic plastic degradation: from machine learning and simulations to wet lab and back

Mme Birgit STRODEL (Research Centre Jülich & CBMN)

Topics

Machine Learning

Communication type

Oral

Abstract

Our research targets plastic waste pollution by enhancing the efficiency of PETase and nylonase enzymes responsible for degrading PET and nylon. Through both standard and advanced molecular dynamics (MD) simulations, we explore how plastic substrates access the active site and align into productive configurations for hydrolysis. This enables the identification of key residues involved in substrate binding, which can be optimized through targeted mutations. Concurrently, we utilize our user-friendly machine learning (ML) tool, TransMEP, which employs transfer learning and Gaussian process regression to predict beneficial mutations based on limited initial experimental enzyme activity data. The synergy of ML and MD approaches leads to the development of enzyme variants, experimentally validated by our collaborators in the wet lab. Feedback from these experiments informs further computational refinement and mutation suggestions. In this presentation, I will discuss our latest results concerning two different PETases and a nylonase.

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#10882

Structural and dynamic biology with integrative approaches

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Topics

N/A

Communication type

Poster

Abstract

Understanding how biological systems function at the molecular level is essential for the rational targeting of disease. The behavior of complex systems often arises from the interplay between structural and dynamic properties. While experimental and computational methods are invaluable for probing protein structure and dynamics, each has limitations. Our lab develops integrative approaches that combine experimental data with molecular dynamics simulations to determine accurate protein structural ensembles. I will illustrate these methods through several applications. First, I will show how our EMMIVox approach determines accurate ensembles from cryo-EM maps [1]. Next, I will describe how we characterize the structure and dynamics of the CyaA toxin by integrating coarse-grained simulations with hydrogen/deuterium exchange mass spectrometry, small-angle X-ray scattering, and single-particle cryo-EM data [2]. Finally, I will demonstrate how structural information from artificial intelligence methods, such as AlphaFold, can be combined with experimental data to generate accurate ensembles of intrinsically disordered proteins [3].

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#10323

Engineering SIRP? conformational plasticity to reveal a cryptic pocket suitable for structure-based drug design

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Topics

Drug Design

Communication type

Oral

Abstract

The protein-protein interaction between Signal Regulatory Protein alpha (SIRPα) and CD47 is a critical immune checkpoint that enables tumor immune escape, making it a key target for cancer immunotherapy. While antibody-based therapies exist, the development of small-molecule inhibitors has been hindered by the flat, featureless binding interface.

Here, we report the discovery of a novel, druggable cryptic pocket within the SIRPα D1 domain, revealed through a structure-based fragment screening campaign using x-ray crystallography. This pocket, defined by three conserved aromatic residues, is only accessible in a conformation that is incompatible with CD47 binding, making it a candidate for structure-based drug design and immune checkpoint inhibitor development.

Through a combination of crystallography, NMR spectroscopy, molecular dynamics simulations, and biophysical assays, we demonstrate that access to this cryptic site is dynamically controlled by a single Gln "gatekeeper" residue. The rotameric state of this residue dictates a conformational equilibrium between a closed state and a ligand-accessible open state. We validated this mechanism by engineering SIRPα mutants to bias this equilibrium. A mutation that locked the pocket in a closed state abolished both CD47 and fragment binding, while mutations that biased the protein toward an open state not only exhibited decreased affinity for CD47 but also significantly improved binding to small-molecule fragments that inhibit the SIRPα-CD47 interaction.

This work uncovers the intrinsic conformational plasticity of SIRPα and establishes a validated, structure-based roadmap for the rational design of a new class of allosteric inhibitors that function by trapping a CD47 non-binding protein conformation.

"Engineering SIRPα conformational plasticity to reveal a cryptic pocket suitable for structure-based drug design", Storder M, et al. & Miller TW. submitted.

#10112

Is Mycobacterial InhA a Suitable Target for Rational Drug Design?

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Topics

Drug Design

Communication type

Oral

Abstract

Tuberculosis is a disease caused by the bacterium Mycobacterium tuberculosis and is the leading cause of death worldwide from a single infectious agent. In 2023, 10.8 million new infections were recorded, and 1.25 million deaths were attributed to this disease. These figures have made tuberculosis a major global public health concern for many years. The World Health Organization has set a goal of reducing the global incidence of the disease by 90% by 2035.

One of the enzymatic targets for tuberculosis treatment is the InhA protein, an NAD-dependent enoyl-acyl carrier protein reductase. This essential enzyme of the fatty acid synthase II system is involved in the biosynthesis of mycolic acids – long-chain fatty acids – specific to mycobacteria. InhA is the target of isoniazid, an anti-tuberculosis drug that has been used since the 1950s. Isoniazid is a prodrug that requires activation by the catalase peroxidase enzyme KatG.

With the emergence of resistance, efforts have been made to develop direct inhibitors of InhA, reinforcing its relevance as a target for the discovery of new anti-tuberculosis drugs. Numerous crystallographic structures—over a hundred in the Protein Data Bank in various forms (apo, holo, and inhibitor-bound complexes) — have been solved as part of these efforts. A crystalline polymorphism has been observed; however, the full structural properties of the protein and its mechanisms of inhibition have not yet been fully elucidated, partly due in part to the molecular plasticity of a key motif: the substrate-binding loop.

A detailed analysis based on available structural data will be presented, with the aim of describing the different families of inhibitors and establishing structural causal relationships (1). Our ongoing work will then be presented, from the design of direct inhibitors based on the diarylether scaffold to the characterisation and evaluation of interactions between these molecules and protein's active site using X-ray crystallography, as well as biological assays on the purified protein and on mycobacterial cultures. In order to facilitate and guide the discovery of new inhibitors, we have implemented methods, such as target-guided kinetic synthesis (KTGS) and dynamic combinatorial chemistry combined with X-ray crystallography (DCX) to enable the pre-organization and detection of fragments/adducts within the target protein.

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#10327

Modelling host-pathogen interactions using multiscale MD simulations: the Mycobacterium tuberculosis case

M Matthieu CHAVENT (CNRS)

Topics

Lipids and membrane proteins

Communication type

Oral

Abstract

Mycobacterium tuberculosis, the pathogen responsible for the infectious disease tuberculosis, is known for its thick and waxy envelope constituted of numerous complex lipids (1). These lipids act both as building blocks to organize the envelope (2,3) and as virulence factors that destabilize the host membrane (4,5) or interact with host-cell receptors (6). I will present our recent works on modeling mycobacterial lipids, their organization in the mycobacterial membranes, and how these lipids can affect host-cell membranes and receptors.

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A structural journey through molecular life and death

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Structural biology is arguably at the height of its time and the combined use of multiple methods resolves problems at atomic level that have long been out of reach. Thereby, solution NMR spectroscopy is ideal to map conformational landscapes and thus connect static structures towards their functional dynamics.
We will go on a journey through the life of biomacromolecules and cells. We start with newly synthesized proteins in eukaryotes. A majority of them is imported into the endoplasmic reticulum (ER), where they are refolded by a network of molecular chaperones. We discover biomolecular condensates as the organizing principle of this chaperone network. We then employ the novel "in cyclo" NMR method to resolve the complete functional cycle of the core protein of this network, the Hsp70 chaperone BiP. BiP undergoes a functional cycle comprised of five states that is regulated by two unique autoinhibiton switches. We conclude our journey with the death of cells. Structural studies of the protein NINJ1 show how it mediates plasma membrane rupture in pyroptosis. We will discuss possible mechanisms of activation and execution, including the "cookie cutter", the "mega-pore" and the "zipper" mechanism.

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#10884

Using a large volume cryo-electron tomography pipeline to study microbial interactions with their environment

Ariane BRIEGEL (Institut Pasteur)

Topics

N/A

Communication type

Poster

Abstract

How are bacterial cells able to actively seek out their preferred environmental niches? How can they evade toxins and predators? How do they interact with phages, each other and their host tissue on a structural level?
Cryo-electron tomography (cryo-ET) is our key research tool to gain insight into the structure and function of the molecular complexes involved in these behaviors. This technique allows us to directly study microbes in their native state at resolutions capable of visualizing individual proteins. However, studying how microbes interact in more complex environments, such as host tissue, is still dauntingly difficult. Here we apply a large volume processing pipeline to study a naturally simplified host microbe system where one organism, the Hawaiian bobtail squid, interacts with only one species of bacteria. Once the squids hatch, the light-producing bacterium Vibrio fischeri in the surrounding seawater migrate toward and into the squid's internal light organ, where they colonize and maintain a light-producing population for the lifetime of the squid. This makes the animal invisible for predators at night, as the animal is camouflaged against the moonlight shining from above. This binary host-microbe interaction is an ideal system to begin to understand the structural interplay between these organisms at the nanoscale.

#10310

STRUCTURAL AND FUNCTIONAL STUDIES of CHIKUNGUNYA NSP1-4 WITH ITS POTENT INHIBITORS

SHRANKHLA BAWARIA (Aix-Marseille University)

Topics

Virology

Communication type

Oral

Abstract

Chikungunya virus is an alphavirus that belongs to the Togaviridae family and is transmitted to humans by Aedes mosquitoes. It possesses a positive sense single stranded RNA genome of about 11.8 kb that contains two open reading frames the first ORF encodes non-structural polyproteins directly from the genomic RNA while the second ORF of about 4.3 kb encodes structural proteins responsible for capsid and envelope formation. During the course of infection, the non-structural polyprotein is cleaved into four proteins: Nsp1 (Capping enzyme), Nsp2 (Helicase/Protease), Nsp3 and Nsp4 (RNA dependent RNA Polymerase, RdRp). Genome replication of Alphavirus occurs in membranous organelles called spherules formed by remodeling of the host cell membrane where Nsp1 acts as an anchorage protein and gates the replication complex into the host cell membrane. Nsp1 has methyltransferase (MTase) and guanylyltransferase (GTase) activities that help in viral RNA capping and make it a promising target for antiviral drugs. There are three series of small molecule inhibitors (MAD, FHA and CHVB) present that has been shown to have significant inhibitory effects based on enzymatic assay using Nsp1 of VEEV or SFV virus (members of alphavirus family). In this study we explore the CHVB series compounds using GTase activities of Nsp1-4 of chikungunya virus. The GTase assay performed on purified complex of CHIKV Nsp1-4 virus confirms the inhibitory effect of CHVB series molecules (34 and 31b; ref: Battisti et.al., European Journal of Medicinal Chemistry 264 (2024)). Furthermore, with the help of cryo-EM based structural studies we identified the interacting pocket of the inhibitor molecule as same as the GTP binding site on the dodecameric assembly of Nsp1 with Nsp4 at the core. These studies confirm that the inhibitor molecule indeed inhibits the GTase activity of Nsp1 of Chikungunya virus.

#10378

Decoding how Chikungunya virus non structural proteins assemble in evolving complexes for seting a sequential program of virus infection

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Topics

Virology

Communication type

Oral

Abstract

Viruses are considered the best cellular biologists; with little genetic information they take control of the cell for self-perpetuation and spread worldwide. Learning how this occurs is extremely difficult because of the large number of cellular factors involved and the multifunctional nature of viral components. Alphaviruses (such as Chikungunya virus) have four non structural proteins which have been studied for decades accumulating loads of information on host interactors, enzymatic and structural data on single domains or partial complexes. However, the functional units of replication and host interactions are macromolecular complexes gathering viral RNA, cell membranes and viral and host proteins. I will present the architecture of in vitro reconstituted full replication complexes showing how the complexes can change protein nsPs composition and stoichiometry in order to form evolving membrane associated replication complexes within replication organelles. I will also present how nsP3, a major interactor with the host, forms tubular scaffolds structuring cytoplasmic Alphagranules which gather host and viral factors into a massif interaction hub. Overall, these data depict the mode of action of Chikungunya nsPs explaining with unprecedented detail how only four four viral proteins manage to deploy multiple functions at different times of the infection and reconfigures the cell cytoplasm landscape.

#9953

Sars-cov-2 Envelope protein–host ZO-1 interaction: from virulence mechanisms to targeted inhibitor discovery

Célia CAILLET-SAGUY (Institut Pasteur)

Topics

Virology

Communication type

Oral

Abstract

The SARS-CoV-2 envelope (E) protein is a critical virulence factor whose highly conserved C-terminal PDZ-binding motif (PBM) mediates deleterious interactions with host PDZ domain-containing proteins, most notably the tight junction scaffolding protein ZO-1 (TJP1). Our recent integrative studies combining in vitro, in vivo, and structural approaches have shed light on how this virus–host interplay contributes to COVID-19 pathogenesis and provided a framework for therapeutic targeting.

Our studies using recombinant SARS-CoV-2 mutants deficient in the E protein PBM demonstrated a significant attenuation of viral replication, cytopathic effect, and virulence in both cellular models and the hamster in vivo system. Importantly, these PBM-deficient viruses induced dramatically reduced airway inflammation, epithelial barrier damage, and clinical symptoms, highlighting the PBM as a virulence determinant. We showed that the viral E protein disrupts the localization and function of ZO-1 in human airway epithelial models, leading to tight junction disorganization and barrier dysfunction. This disruption is associated with exacerbated inflammatory responses.

In order to elucidate the molecular basis of this host-pathogen interface and explore new therapeutic avenues, we have integrated high-resolution structural biology with molecular screening. We solved the crystal structure of the ZO-1 PDZ2 domain in complex with the SARS-CoV-2 E protein PBM peptide, showing the canonical binding mode and a domain-swapped dimer conformation of ZO-1. These insights clarified the structural determinants governing PBM recognition and laid the foundation for inhibitor screening. Using a homogeneous time-resolved fluorescence (HTRF) assay and a library of 1,000 protein–protein interaction modulators, we identified and characterized several small molecules that disrupt the E–ZO-1 PDZ2 interaction. Docking and structural analyses delineated two classes of inhibitors: those competitively targeting the PBM binding groove, and others interfering with ZO-1 dimerization. Most notably, one hit compound exhibited significant antiviral activity in a nanoluciferase-reporter SARS-CoV-2 infection model, validating the therapeutic potential of this structural interface.

Our findings support that the pathogenic effect of the SARS-CoV-2 E protein is closely associated with its PBM-dependent interaction with host ZO-1, leading to epithelial barrier disruption and inflammatory responses. The integration of virological, structural, and chemical screening data approaches illustrates how combining complementary methods can enhance our understanding of virus–host interactions and inform structure-based therapeutic strategies. Modulating the E–ZO-1 interface may represent a promising avenue to limit SARS-CoV-2–induced barrier damage, and could inspire approaches against other viruses relying on similar PBM–PDZ interactions.

#10885

Mechanistic insight into the step-by-step biosynthesis of glycosaminoglycans

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Topics

N/A

Communication type

Poster

Abstract

Glycosaminoglycan (GAG) chains are complex polysaccharides covalently attached to core proteins and located at the cell surface and within the extracellular matrix, where they regulate a wide range of physiological and pathological processes. GAG biosynthesis is a stepwise process that occurs in the Golgi lumen and relies on the finely tuned interplay among more than 30 different glycosyltransferases and glycan-modifying enzymes. In this presentation, I will summarize our recent work focusing on various aspects of GAG biosynthesis, with particular emphasis on the molecular characterization of heterodimeric enzyme complexes that catalyze heparan sulfate and chondroitin sulfate chain polymerization.

#10050

Moss BRCA2 interacts with recombinases and ssDNA in DNA repair

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Topics

Cancer

Communication type

Oral

Abstract

Double Strand Break (DSB) repair is crucial to ensure the genome integrity. In mitotic S-phase and in meiosis, it is achieved by a process known as Homologous Recombination (HR). Central to this process is the BRCA2 protein. It interacts with RAD51 and DMC1 strand exchange proteins (recombinases) by the intermediate of conserved A- and P-motifs. A-motifs, found in the eight BRC repeats of the central region of human BRCA2, compete with the regions of RAD51 and DMC1 that are responsible for self-oligomerization. Thus, the BRC repeats bind to monomeric recombinases. Conversely, two P-motifs were identified in human BRCA2. They interact with oligomeric RAD51 and DMC1, respectively, and contribute to the stabilization of the recombinogenic nucleoprotein filaments[A].

The model plant organism Physcomitrium patens serves as an insightful model system for studying HR, due to its relatively simple genome architecture and high rate of HR (>90% compared to less than 1% for other plants). We have identified a moss protein that share sequence homologies with human BRCA2. This PpBRCA2 differs from human BRCA2 as it is about 10 times shorter (391 aa), and lacks a folded DNA-binding domain. We have also identified recombinases: PpRAD51-1, PpRAD51-2 and PpDMC1, which share about 70% and 60% sequence identity with human recombinases, respectively. The presence of these proteins suggests that similar HR mechanisms exist between the two species. Here, I show that PpBRCA2 is entirely disordered. It contains an N-terminal positively charged region with two putative P-motifs, and a C-terminal negatively charged region with four A-motifs located in BRC repeats. I present biochemical evidence that PpBRCA2 can bind to PpRAD51 through its A-motifs and stabilize PpRAD51 filaments through one of its P-motifs. Surpringly, no interaction was observed with PpDMC1 so far, meaning that specific HR mechanisms could differ between moss and human. I also reveal evidence for an interaction between the N-terminal region of PpBRCA2 and ssDNA, which might play a role similar to that of the disordered regions of human BRCA2 binding to DNA[B,C].

Meanwhile, novel partners of PpBRCA2 were identified by co-immunoprecipitation and mass spectrometry by the team of R. Kumar. I analyzed their results using AlphaFold-Multimer and an interaction prediction approach with delimited peptides of PpBRCA2. I found that a direct interaction is predicted with a reasonable confidence score between PpBRCA2 and PpRADA, a protein homologous to RadA in bacteria. In Arabidopsis thaliana, this protein was found to possess the same activities as bacterial RadA[D].

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#10318

The role of the WRN exonuclease/helicase in coordinating DNA repair and replication pathways, and their connection to premature aging syndromes

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Topics

Cancer

Communication type

Oral

Abstract

DNA replication and repair ensure the faithful transmission of genetic information. DNA double-strand breaks (DSBs) and lesions affecting DNA replication machinery are among the most cytotoxic forms of DNA damage.

We previously reported structural and functional analyses of the human Ku70/Ku80 heterodimer (Ku) in DSB repair. Specifically, we characterized how Ku contributes to the assembly of the ~1 MDa DSB repair complex, notably through its interactions with Ku-binding motifs (KBMs) (1–4). Furthermore, we demonstrated that Ku also plays a role in the restart of replication forks stalled by DNA lesions, by recruiting the exonuclease/helicase WRN.

WRN interacts with Ku through KBMs located at both termini of the protein. To further investigate this interaction, we purified several constructs of the full-length WRN protein (1,432 amino acids). We determined cryo-EM and X-ray crystal structures of WRN domains in complex with Ku. Using biophysical techniques, including biolayer interferometry, isothermal titration calorimetry, and mass photometry, we showed that WRN constructs bind Ku in the micromolar range, and that full-length WRN forms a dimer in solution. We validated these in vitro findings in a cellular context using micro-irradiation and genotoxic stress assays.

Overall, our study enhances the understanding of WRN's dual role in DNA repair and replication pathways and provides new insights into the molecular basis of syndromes associated with WRN deficiency.

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#10330

Novel Regulatory Mechanisms of the Pseudokinase KSR in the RAS-ERK Pathway

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Topics

Cancer

Communication type

Oral

Abstract

RAF kinases function to relay signals within the RAS-RAF-MEK-ERK (RAS/ERK) cascade. Unbridled signaling through the RAS/ERK pathway caused by activating mutations in RAS and RAF is a major driver of tumor formation (30% of all cancers). RAF activation is achieved by the adoption of a dimer configuration of its kinase domain with the pseudokinase KSR, which in turn allosterically transactivates RAF, leading to MEK phosphorylation(1). We previously showed that MEK binding to the pseudokinase KSR induces KSR-RAF heterodimerization and potentiates RAF activation(2).

Two other proteins, the scaffold protein CNK and HYP, were identified to potentiate the ability of the pseudokinase KSR to transactivate RAF(3). Here, we aimed at understanding the molecular mechanism by which CNK and HYP cooperate with the pseudokinase KSR to transactivate RAF and induce the activation of the RAS/ERK signaling cascade.

To this aim, we combined structural biology approaches, such as X-ray crystallography and cryo-EM, with biochemical and functional characterization of the CNK:HYP:KSR:MEK protein complex. This study revealed new insights into how the pseudokinase KSR plays a pivotal role in the multi-layer mechanism of regulation of the RAS/ERK signaling pathway(4).

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#10886

Quaternary structure and DNA recognition of a toxin-antitoxin-chaperone system of Mycobacterium tuberculosis

Lionel MOUREY (IPBS / CNRS - Université de Toulouse)

Topics

N/A

Communication type

Poster

Abstract

Bacterial toxin-antitoxin (TA) systems are stress response elements composed of a stable toxin that forms an inactive complex with its cognate antitoxin. In response to stress conditions, the antitoxin is degraded by proteases and the free toxin can then target cellular processes. The toxin-antitoxin-chaperone (TAC) system discovered in Mycobacterium tuberculosis is an atypical TA system tightly controlled by a molecular chaperone (1). We contributed to the identification of the role played by the C-terminal extension of the antitoxin, which we called ChAD (for 'chaperon addiction'), for the interaction with the chaperone and which makes it dependent on the latter (2). In particular, we studied the interaction between 13-mer peptides flanking the ChAD sequence of the antitoxin and the chaperone (2,3), and resolved the structure of one of these complexes (4). More recently, it has been shown that the TAC toxin is a ribonuclease that cleaves mRNAs at specific position during translation and the structure of the ribosome-associated toxin with one of its preferred mRNA substrates was solved by cryo-EM as well as the structure of the toxin alone using X-ray crystallography (5). We are currently focusing on resolving the structure of several multiprotein and ribonucleic complexes of the TAC system, as well as their characterization in solution (6), which will be presented.

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#10354

Unique structural features of the human Kir2.1 channel in two different conformational states unveiled by cryo-electron microscopy

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Topics

Lipids and membrane proteins

Communication type

Oral

Abstract

Kir2.1 belongs to a family of inward rectifier potassium (K+) channels that selectively control the permeation of K+ at the cell membrane of a variety of tissues. Expressed especially in cardiomyocytes, Kir2.1 contributes to the establishment and maintenance of the resting membrane potential and action potential plateau.

Although Kir channels are constitutively active, their gating is modulated by intracellular ligands, such as divalent cations and polyamines, but mainly by the membrane lipid phosphatidylinositol 4,5-bisphosphate (PIP2), that binds directly to the channel and allows for its electrical activity. Recently, an endogenous cardiac miRNA (miR1) was discovered by our collaborators as a physiological regulator that physically binds to Kir2.1 and suppresses K+ current (Yang et al., Circulation 2021). However, how the channel gates in response to modulators is only beginning to be understood.

Having solved the first structure of the human Kir2.1 channel (Fernandes et al., Science Advances 2022), here we present the two complex structures solved by cryo-EM; in the activatory PIP2-bound state (3.26 Å) and the inhibited miR1-bound state (2.75 Å). Our results reveal unique structural differences between the two conformations. Given that both ligands have distnict binding sites, our surface plasmon resonance interestingly demonstrate a competition between the two modulators, indicating a preferential binding to a specific conformational state.

These findings shed light on the so far obscure gating molecular mechanism of human Kir channels and the effect of modulators, such as PIP2 and miR1, on the associated conformational rearrangements that are vital for the regulation of the cardiac action potential.

#10249

Condensation of an intrinsically disordered translation factor eIF4B: from in vitro to in-cell

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Topics

RNA/DNA

Communication type

Oral

Abstract

Emerging research suggests that the formation of biomolecular condensates, often mediated by phase separation of intrinsically disordered proteins (IDPs) and nucleic acids, is essential for regulating fundamental physiological processes and is also implicated in neurodegenerative diseases. To discern the molecular mechanisms behind the formation of biomolecular condensates, a deep understanding of condensation at mesoscale and underlying molecular processes at nanoscale is highly important both in vitro and ultimately in living cells. To this end, my project focuses on in vitro and in-cell characterization of condensation of eukaryotic translation initiation factor 4B (eIF4B), an intrinsically disordered translation factor implicated in regulation of protein synthesis and formation of stress granules. In this work, the co-localization of endogenous eIF4B in stress granules (SGs), along with G3BP1, a SG marker protein, is first confirmed using an immunofluorescence (IF) assay. Remarkably, eIF4B-positive foci are also found to form in U2OS G3BP1/G3BP2 single as well as double knockout cells, while G3BP1/G3BP2-positive foci are detected in U2OS eIF4B knockout cells upon stress induction. These results highlight that the liquid–liquid phase separation of eIF4B inside the cell is independent of G3BP1/G3BP2-mediated SG assembly and vice versa. While probing the molecular basis of eIF4B phase separation in vitro, our group has previously shown that DRYG region of eIF4B with its large fraction of tyrosine residues, is essential for its condensation in vitro. In line with this observation, my results show that SG induction in cells expressing eIF4B variants exhibits a significant reduction in SG accumulation propensity for eIF4B variant, where tyrosine residues are replaced by serine residues in the DRYG region. Apart from aromatic interactions, phosphorylation has also been found to play a key role in eIF4B phase separation. The eIF4B dual phosphomimetic mutant S422E/S406E exhibits reduced affinity for self-association at the oligomeric level under low ionic strength, forming smaller protein clusters than the wild type and displays a significant decrease in phase separation propensity at the mesoscale level. It would be further intriguing to observe how the phase behavior of this dual phosphomimetic differs from that of the wild type within the cellular environment. In addition to the above-mentioned findings, my single-molecule experiments enable characterizing the distinct intra- and inter-molecular dynamics of eIF4B and in the presence of RNA, which can help in rationalizing the condensation behavior of the protein.

Elucidating the interaction mechanisms of intrinsically disordered proteins at atomic resolution

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Intrinsically disordered proteins (IDPs) display a remarkably versatile interaction landscape, characterized by coupled folding and binding, highly dynamic complexes, and multivalent interactions. By combining NMR spectroscopy and X-ray crystallography, we elucidate the binding mechanisms of IDPs at atomic resolution. In particular, we map the dynamics of IDPs within complexes, we visualize the folding trajectories of IDPs that undergo structural transitions upon partner binding, and we study promiscuous IDPs that interact with multiple partners, adopting distinct conformations. Our work emphasizes the complexity of the IDP interaction landscape and highlights the expanding role of NMR spectroscopy in characterizing these highly dynamic systems.

#10887

N-terminal capping tunes fibrillation and cytotoxicity of Staphylococcus aureus PSM?3

Marion MATHELIÉ-GUINLET (CBMN, CNRS, Univ. Bordeaux)

Topics

N/A

Communication type

Poster

Abstract

The virulence of Staphylococcus aureus, a multi-drug resistant pathogen, is tightly linked to the expression of phenol-soluble modulins (PSMs), notably PSMα3, the most cytotoxic member of PSMs which self-assembles into unique cross-α amyloid-like fibrils[1.] Here, we investigate how N-terminal capping influences PSMα3's aggregation behavior and cytotoxic potential under near-physiological conditions.

Using Thioflavin T (ThT) fluorescence spectroscopy, NMR, and TEM, we compared formylated (f-) and acetylated (ac-) PSMα3 forms. The more hydrophobic ac-PSMα3 exhibits reduced ThT binding and preserves a greater proportion of soluble species, with fibrils observed at late aggregation stages that morphologically differ from f-PSMα3. Importantly, this functionally correlates with significantly reduced cytotoxicity towards HEK293 cells, in a serum deprived medium, both in a time- and concentration-dependent manner. Using Laurdan fluorescence and Atomic Force Microscopy, we also demonstrated that only the intermediate species led to membrane fluidification, a disruptive mechanism that was further validated in vitro using model membranes of controlled lipid compositions[2]. Such in vitro studies finally revealed that membrane properties - particularly fluidity and lipid segregation - are key determinants for PSMα3 activity[3]. Overall, our results demonstrate that the N-terminal capping critically modulate PSMα3 aggregation and cytotoxicity via membrane permeation4, thus offering new insights into S. aureus pathogenicity.

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#10337

Molecular bases for functional disruption of synapse by autoantibodies in limbic encephalitis

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Topics

Neurobiology

Communication type

Oral

Abstract

More than 50 million people world-wide have epilepsy, and ca. 30% of them continue to experience seizures despite medical management. In addition to symptoms of altered consciousness, fever, and focal neurological deficits, patients suffering from autoimmune limbic encephalitis, a particular form of epilepsy, often present neuropsychiatric manifestations and seizures. These patients generate autoantibodies that target neuronal cell surface antigens, including neurotransmitter receptors, ion channels, or related proteins. Of these, the secreted Leucine-rich Glioma-Inactivated protein 1 (LGI1), consists of an N-terminal LRR domain and a C-terminal EPTP domain. At excitatory synapses, association of a LGI1 dimer with membrane-tethered ADAM proteins forms a trans-synaptic complex contributing to synapse cohesiveness and functioning.

Previously, using a culture model we showed that anti-LGI1 autoantibodies are genetically heterogenous and can be classified into three groups: two that target the LRR domain non-competitively to each other, and one that targets the EPTP domain [1-3]. To identify the molecular bases of autoantibody binding to LGI1, we carried out molecular mapping of their paratope-epitope interfaces and recording of their binding parameters.

To this end, we generated recombinant forms of LGI1, LGI1 domains, and ADAM. From a collection of patient-derived monoclonal antibodies (IgGs), we selected the most representative of them using LGI1 tethered to a SPR sensor chip, and generated recombinant forms of their Fab fragments, of which we determined the binding parameters. We solved crystal structures of all Fabs in their unbound state, and several Fab-LRR complexes. We also solved negative-stain and cryo-EM structures of binary, ternary and quaternary complexes involving various combinations of LGI1 or its domains, Fabs, and ADAM. And most of the possible complexes were modelled using AlphaFold3.

This study led us gather a comprehensive set of complementary data shedding light on how sequestering of the LGI1 LRR or EPTP domain by autoantibodies precludes formation of a functional LGI1-ADAM dimer across neuronal synapses and disrupts the presynaptic and postsynaptic signalling events associated with synaptic functioning. These data provide molecular templates for the design of therapeutic strategies aimed at alleviating the pathogenic effects of anti-LGI1 autoantibodies in limbic encephalitis patients.

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#10341

Role of monomers, Dimers, and Oligomers of Pyroglutamate-Modified ?-Synuclein Fragments in fibril assembly.

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Topics

Neurobiology

Communication type

Oral

Abstract

α-Synuclein (aSyn) aggregation represents a key event in the neurodegenerative cascade of synucleinopathies. Neosynthesized aSyn is an intrinsically disordered protein, whose structural flexibility allows it either to adopt α-helical conformations, relevant for physiological functions at presynaptic vesicles, or to form β-strand-rich aggregates, leading to toxic oligomers. This relation between structure, function, and toxicity can be influenced by post-translational modifications such as the recently identified glutaminyl cyclase-catalyzed pyroglutamate (pE) modification. Here, we investigate the structural characteristics of monomeric, dimeric, and oligomeric states of Full length (FL) and N-terminal truncated, pE-modified aSyn variants, pE24-, pE62-, and pE79-aSyn by a complementary panel of biophysical approaches including DLS, SEC-MALS, SRCD, SEC-SAXS and AUC. This orthogonal strategy provided detailed information on the secondary structure content, the overall size, and low-resolution shape of the monomeric FL-aSyn and pE-aSyn variants. Rg values for the monomers were smaller than expected for a completely unfolded polypeptide (52 Å) but larger than the theoretical Rg of a globular protein (15.1 Å), reflecting a possible folding or compression of the pE-aSyn. This finding was confirmed by intrinsic viscosity and sedimentation velocity measurements. Furthermore, the integrative approach combining AUC and SEC prior to SAXS analysis allowed to separatethe different aggregation states of FL-aSyn and of the pE-aSyn variants. Compared to the FL-aSyn monomer, the size of the dimer did not increase significantly while the frictional ratio strongly decreased. The comparative analysis of these values indicates the existence of an intermediate state for the dimer, between the mostly unfolded and the folded states, allowing us to suggest, for the first time, a SAXS data-based model of the dimeric state of FL-aSyn in a head to tail conformation. To understand the arrangement of further assembly intermediates, such as tetramers and hexamers detected by AUC, a series of models compatible with our available hydrodynamic results were generated by bead assembly modeling. Overall, our data show that all models are in favour of an elongation by stacking of dimeric units. To conclude, our work results in an unique model of fibril formation showing the first step of the process, and reflecting the aggregation-prone properties of the pE-modified aSyn variants.

This project has received funding from the European Union's Horizon 2020 Research and Innovation Programme under grant agreement No. 101004806 (MOSBRI)

#10255

Understanding Tau’s Chemical code: microtubules, lipid membranes, and phosphorylation

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Topics

Neurobiology

Communication type

Oral

Abstract

Tau is an intrinsically disordered neuronal protein that aggregates into β-sheet-rich amyloid fibrils in Alzheimer’s disease (AD). Using solid-state NMR and cryo-EM, we investigated how tau’s sequence, posttranslational modifications, and membrane interactions govern its conformational transitions and aggregation.

Tau’s microtubule-binding domain contains four repeats and a pseudorepeat (R′). We show that R′ plays a dominant role in binding microtubules via electrostatic interactions, remaining immobilized while R4 becomes dynamic, especially with unstable microtubules.

Tau’s membrane interactions further influence its aggregation. On high-curvature, cholesterol-rich vesicles, tau adopts the AD-relevant paired helical filament (PHF) fold and inserts into the membrane interface. At lower protein concentrations, lipids perturb the fibril structure without fully altering it. Notably, removal of phospho-mimetic mutations leads to different β-sheet conformations, indicating phosphorylation exerts a stronger influence than membranes on tau’s structure.

To understand aggregation, we examined fibrils formed by full-length and truncated tau carrying AD-relevant phospho-mimetic mutations. PHF1 and AT8 phospho-patterns produce distinct fibril cores, spanning R2–R4 or R3–C-terminus, respectively. Truncation at D421, an early AD marker, drives aggregation into a structure nearly identical to PHF1-mutant fibrils. However, combining ΔD421 with AT8-mimetic mutations yields a new fibril that includes the typically disordered C-terminal domain, suggesting these modifications are mutually exclusive in disease.

Overall, our findings show how tau’s structural landscape is shaped by its modular sequence, phosphorylation state, and membrane environment, factors that cooperatively control its physiological and pathological assembly.

Annexes

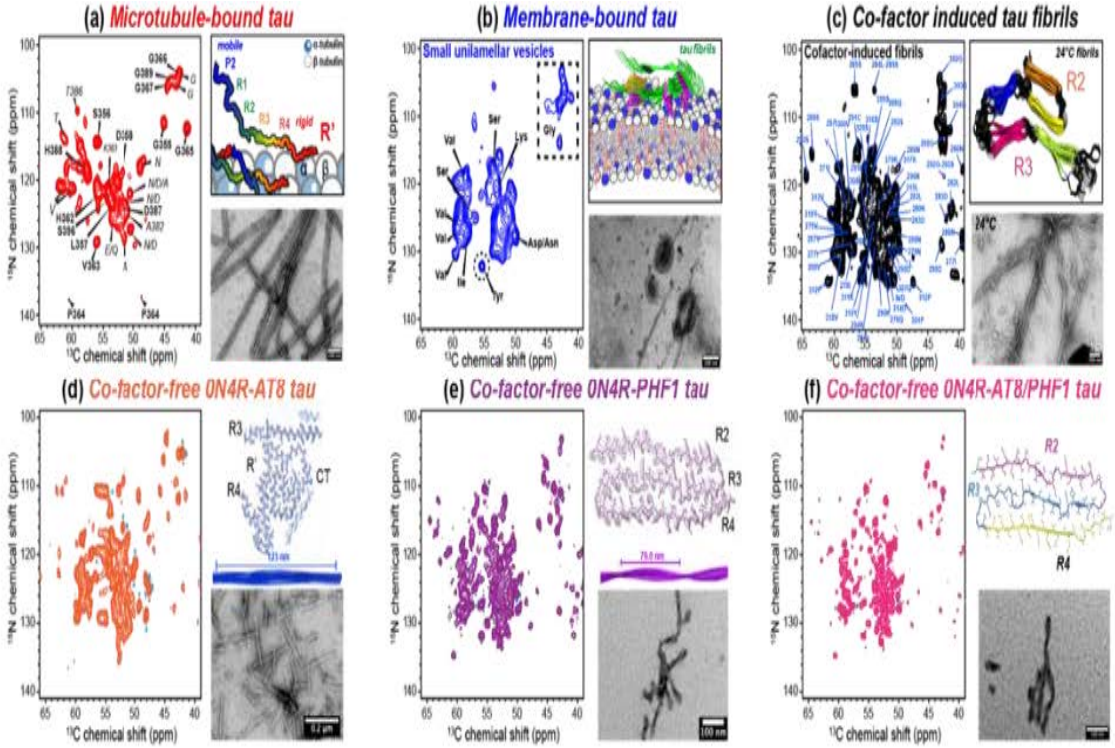

Figure 1: Figure 1. (a-f) 2D NCA correlation spectrum (left) and TEM image (bottom right) of multiple tau samples: tau bound to microtubules, tau bound to SUVs, heparin-induced tau(198-299) fibrils, co-factor free pseudophosphorylated 0N4R-AT8-3E tau mutant fi

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Figure 2

#10888

Bridging Solution and Solid-State NMR to Elucidate Hepatitis D Virus Protein-RNA Interactions

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Topics

N/A

Communication type

Poster

Abstract

Hepatitis D virus (HDV) replication and packaging rely on interactions between its RNA genome and the delta antigen (HDAg). The small antigen, S-HDAg, comprises an N-terminal assembly domain, which forms an octameric structure, and a flexible C-terminal domain. The full-length protein is insoluble, posing major challenges for structural studies. Using an integrative structural biology approach combining solution and solid-state NMR, ITC, AFM, and EMSA, we characterized the structure and RNA-binding properties of S-HDAg domains. Solid-state NMR revealed that the assembly domain is conserved in the full-length protein and that it represents the only rigid part of the protein (Yang et al., 2024). Solution NMR showed that the C-terminal domain features two intrinsically disordered regions flanking a helix-loop-helix motif that mediates RNA binding (Yang et al., 2025). Biophysical analyses indicate that ~200 S-HDAg molecules associate with a single viral RNA with submicromolar affinity. These findings provide the molecular basis for understanding HDV ribonucleoprotein assembly, and highlights the strength of NMR and combination of techniques in unraveling protein-RNA interactions, particularly in the context of large viral systems.

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#10314

Balancing activation and inhibition of immune receptors: AlphaFold-guided structural analysis of the paired NLRs RGA4 and RGA5

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Topics

Plants

Communication type

Oral

Abstract

Nucleotide-binding and Leucine-rich Repeat domain (NLR) proteins are intracellular receptors that play a central role in plant and animal immunity. In plants, they form the most abundant class of resistance proteins. They typically detect pathogen-secreted proteins called effectors and trigger downstream signaling that leads to the death of the infected cell. NLRs exhibit a conserved modular architecture, characterized by a central NB-ARC domain acting as a conformational switch in response to effector recognition. The activation model of plant NLR is based on the cryo-EM structures of ZAR1, which revealed that singleton NLRs switch their NB-ARC domain from an 'OFF' to an 'ON' state, stabilized by an ADP/ATP exchange. This transition allows assembly into a multimeric complex called resistosome that in the case of ZAR1 forms a pentameric calcium channel. Although this general activation model is expected to be conserved, recent structural studies revealed how diverse and complex these proteins are. Deciphering this natural diversity is critical for elucidating how plants deploy immunity against pathogens and for engineering durable disease resistance in crops.

In contrast with singleton NLRs, the RGA4 and RGA5 immune receptors from rice (Oryza sativa) are paired NLRs that work together to confer resistance to the blast disease caused by the fungus Magnaporthe oryzae. RGA5 functions as a sensor that, in the absence of fungal effectors, inhibits the auto-activity of the executor RGA4. RGA5 possesses a non-canonical C-terminal HMA domain that directly binds the AVR-Pia or AVR1-CO39 effectors, lifting RGA5 repression on RGA4. So far the structural basis of the regulation mechanism for such paired NLRs is unknown.

Using different in silico tools and versions of AlphaFold (AF), we modelled RGA4 and RGA5 as monomers and as various heterodimeric and multimeric complexes. In spite of their close sequence similarity, RGA4 was always modeled with its NB-ARC domain in the ON state conformation, as might be expected for an auto-active NLR, whereas RGA5 was consistently predicted in the OFF state. This observation suggests that this NLR pair may not require a conformational switch to function. More over in the models of the RGA4-RGA5 inhibition complex, RGA5 occupies one of the RGA4 oligomerization interfaces, suggesting that RGA5 could compete with the auto-assembly of RGA4 units. To challenge these predictions, we conducted site-directed mutagenesis at specific residues involved in the formation of the auto-active RGA4 resistosome and/or inhibition complex. Mutants were transiently expressed in Nicotiana benthamiana to assess their activity and repression levels, and to validate the loss of interactions through co-immunoprecipitation. Overall, our results validate the AF-predicted models and support an inhibition mechanism where RGA5 engages RGA4 through hetero-dimeric interactions that occlude the oligomerization surfaces required for resistosome assembly. This steric blockage would prevent RGA4 from adopting the multimeric conformation characteristic of active NLR complexes. These findings provide experimental evidence that structural inhibition in paired NLRs can be mediated by direct competition at oligomerization interfaces and may not rely on an ADP/ATP exchange for balancing the activation/inhibition process,offering a mechanistic contrast to the singleton paradigm.

Annexes

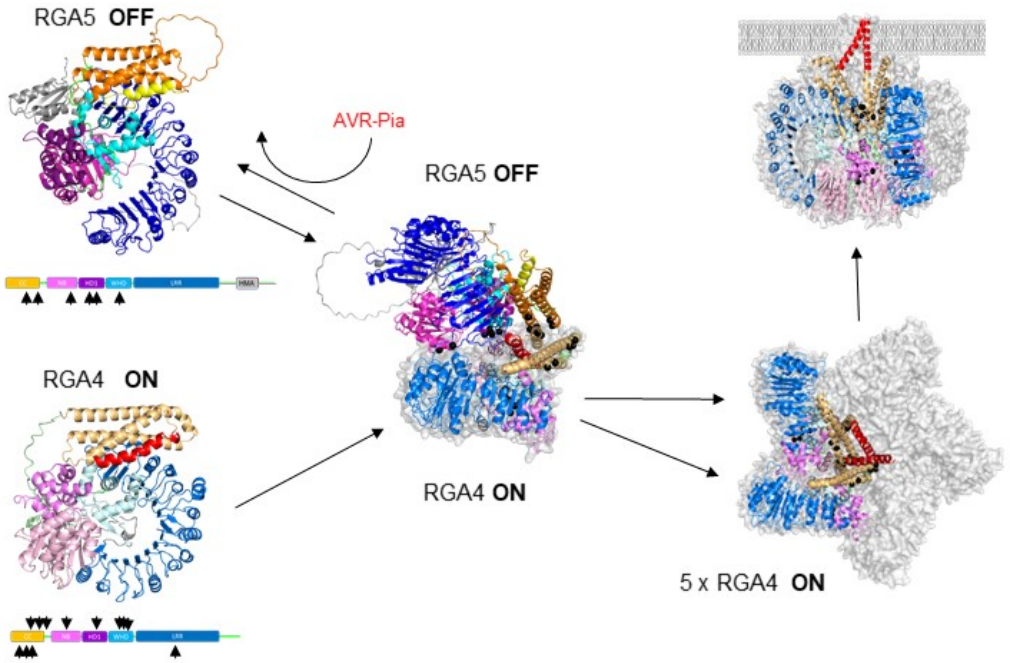

Figure 1

#10371

Structure of a photosystem II-FCPII supercomplex from a haptophyte reveals a distinct antenna organization

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Topics

Plants

Communication type

Oral

Abstract

Haptophytes are unicellular algae that produce 30 to 50% of biomass in oceans. Among haptophytes, a subset named coccolithophores is characterized by calcified scales. Despite the importance of coccolithophores in global carbon fixation and CaCO3 production, their energy conversion system is still poorly known. Here we report a cryo-electron microscopic structure of photosystem II (PSII)-fucoxanthin chlorophyll c-binding protein (FCPII) supercomplex from Chyrostila roscoffensis, a representative of coccolithophores. This complex has two sets of six dimeric and monomeric FCPIIs, with distinct orientations. Interfaces of both FCPII/FCPII and FCPII/core differ from previously reported. We also determine the sequence of Psb36, a subunit previously found in diatoms and red algae. The principal excitation energy transfer (EET) pathways involve mainly 5 FCPIIs, where one FCPII monomer mediates EET to CP47. Our findings provide a solid structural basis for EET and energy dissipation pathways occurring in coccolithophores.

#10273

Quantitative lipid analysis of brain, myelin-enriched and corpus callosum fractions in mice, by multinuclear NMR and mass spectrometry

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Topics

Lipids and membrane proteins

Communication type

Oral

Abstract

Myelin is a key component of the central nervous system. It is a lipid and protein lamellar membrane structure that tightly envelops the axon in a concentric fashion (cochlear structure). Present in the central and peripheral nervous systems of vertebrates, it gives its name to white matter due to its high concentration (80%) of phospholipids, glycosphingolipids and cholesterol. It plays a key role in signal transmission and neuron survival in the central nervous system, and its loss or lack of integrity can lead to a number of neurodegenerative diseases in particular Multiple Sclerosis Although myelin preserves several aspects of biological lipid bilayers, its peculiar membrane organization is still not fully understood. To decipher compositional and dynamics properties of myelin, we proposed a combined NMR and mass spectrometry approach to quantitatively investigate lipid composition. We compared various samples ranting from mice whole brain, corpus callosum (the largest commissural white matter bundle in the brain,) and extracted myelin-enriched fraction to study their lipid composition. By comparing control samples, specimens extracted from animals in pathological conditions and in vitro reconstituted lipid membranes, we provided a detailed description of lipid dynamics.
* ANR ULTIMO ANR-22-CE18-0041.

#10334

Time-resolved cryo-EM to visualize biomolecules in action

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Topics

Methods

Communication type

Oral

Abstract

Time-resolved cryo-electron microscopy (cryo-EM) is a technique that allows to take structural “snapshots” of biomolecules in action (during a reaction, in real time). Its principle is to start the reaction of interest with a trigger and then stop it by vitrification on a cryo-EM grid after a well-defined, yet variable time delay to “freeze-trap” reaction intermediates.

Some of the triggers can be on-grid or microfluidic mixing of two or more reaction partners, a light pulse to directly activate photosensitive proteins or photolabile “caged” compounds on the grid, or using a laser to melt an already vitrified sample for a short time (µs). When the time between the trigger and vitrification is longer than a few seconds, the grids can be prepared using conventional methods. However, for “fast” reactions (< 1 s), and some triggering methods, a specialized experimental setup is needed.

Time-resolved cryo-EM can be complementary to time-resolved X-ray crystallography: the latter allows to access a wide range of time delays (from sub-ps at XFELs to ms at synchrotrons) but is limited for systems where large domain movements are restricted by crystal packing. Time-resolved cryo-EM is limited to “slower” time delays (from tens of ms to seconds) but since there is no need for crystals, larger conformational changes can be studied.

Here we present an example of this complementarity, in the study of the later stages of the photoreaction of the bacterial transcription factor CarH. This protein is the most studied member of a family of photoreceptors that use vitamin B12 as a chromophore. In the dark, CarH forms a tetramer that binds DNA and blocks the transcription of genes involved in carotenoid biosynthesis. When exposed to light, the tetramer dissociates in an irreversible reaction, and does not bind DNA anymore, allowing the production of carotenoids, which protect bacteria from photodamage. The study of the CarH photoreaction by time-resolved X-ray crystallography is limited to 10 ms after illumination because large domain movements disrupts the crystal packing for longer pump-probe delay.

Time-resolved cryo-EM offered us a way to freeze-trap intermediate states at later stages of the photoreaction and determine their high-resolution structures. We expressed and purified CarH (full-length, truncated, and in complex with two DNA fragments), confirmed its photoactivity on grids, and obtained high-resolution cryo-EM structures of all these samples in their initial “dark” state. We also successfully prepared cryo-EM samples of the CarH photoreceptor in a time-resolved way with trigger-freeze delays between 50 and 600 ms. In particular, we found a previously unobserved reaction intermediate of CarH, adding new details to our understanding of its reaction mechanism.

Mechanisms of activation and diversification of the bacterial cellulose synthase

Petya V Krasteva

Cellulose — or the unbranched chains of β-1,4-linked D-glucose moieties — represents the major component of the plant cell wall and thus the most abundant biopolymer on Earth. In addition, cellulose is secreted by many and diverse viral, prokaryotic, and eukaryotic organisms, where it most commonly confers resistance to environmental stress and/or contributes to the establishment of multicellularity. Bacteria, in particular, have co-opted secreted cellulosic polymers as extracellular biofilm components, which - together with extracellular DNA and proteinaceous fimbriae, such as adhesins, amyloid curli, flagellar filaments and/or type IV pili - lead to the development of a complex, spatially, and functionally differentiated matrix for macrocolony homeostasis. Bacterial cellulose (BC) is polymerized from a pre-energized UDP-glucose substrate and extruded through the (inner) cell membrane by processive multidomain synthases, which assemble into complex multicomponent Bcs secretion systems and are most often under the control of the intracellular second messenger c-di-GMP. The secreted cellulosic chains can be decorated with additional chemical groups or can pack with various degrees of crystallinity depending on dedicated enzymatic complexes and/or cytoskeletal scaffolds. Here I will present our recent progress in the understanding of synthase-dependent EPS biogenesis with focus on common and idiosyncratic molecular mechanisms of synthase activation and polymer diversification across multiple biofilm-promoting cellulose secretion systems.

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@ corresponding author * equal contribution

POSTER PRESENTATIONS

(In the order of submissions)

#10044

A Coiled Coil Module Strategy for High-Resolution Cryo-EM Structures of Small Proteins for Drug Discovery

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Topics

Methods

Communication type

Poster

Abstract

Electron cryo-microscopy (Cryo-EM) has traditionally been used for structural determination of proteins larger than 50 kDa. Recently, various approaches, such as fusion to a scaffold or the use of DARPins-cages, have been developed to extend its application to smaller proteins. In this study, we determined the structure of the small protein target kRasG12C by fusing it to the coiled-coil motif APH2, which is targeted by several nanobodies. This method enabled us to achieve a structure with atomic details at a resolution of 3.7 Å. The kRasG12C structure was bound to the inhibitor drug MRTX849 and GDP, both clearly visible in the density map. This method is advantageous due to its ease of setup and applicability to other targets. Additionally, we investigated several other techniques that can be applied to small proteins, regardless of the presence of a terminal helix. These advancements demonstrate the potential of Cryo-EM for detailed structural analysis of a wide range of protein targets, extending cryo-E

#10051

Structural basis for NONO specific modification by the a-chloroacetamide compound (R)-SKBG-1

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Topics

Cancer

Communication type

Oral

Abstract

Among the many proteins involved in cancer progression an increasing number of RNA Binding Proteins (RBPs) are central to the function of a cell and tightly associated to genetic diseases as well as cancer appearance and progression. In a recent study, small molecule inhibitors have been identified as targeting NONO, a RBP known to be involved in mRNA splicing, DNA repair and membraneless organelles stability. Here we report the molecular basis of NONO-targeting by the a-chloroacetamide (R)-SKBG-1. We explore the specific binding and enantiomer specificity of NONO towards (R)-SKBG-1 using mass spectrometry and structure determination.

We have determined the crystal structure of (R)-SKBG-1-bound to NONO homodimer. This study sheds light on the conformational plasticity of (R)-SKBG-1 when covalently bound to NONO. Altogether these results give an experimental rationale for ligand modification and optimization in a future use as a drug against cancer.

#10098

Decoding nuclear access: importin-mediated trafficking of the aryl hydrocarbon receptor

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Topics

Other

Communication type

Oral

Abstract

The aryl hydrocarbon receptor (AHR) is a ligand-dependent transcription factor known to mediate gene expression in response to a wide array of exogenous and endogenous ligands, including chemical pollutants, natural compounds, and metabolic by-products. Upon activation by its ligands, AHR translocates from the cytoplasm to the nucleus, where it orchestrates transcriptional responses by binding to specific DNA elements. Although nuclear translocation is essential for AHR activity, the molecular basis of its import has remained unclear.

To elucidate these mechanisms, we conducted a comprehensive study of the interactions between AHR and the nuclear import machinery. Specifically, we focused on the importin (karyopherin) family, which mediates the transport of cargo proteins through the nuclear pore complex. We successfully expressed and purified all seven members of the human importin alpha (KPNA1–KPNA7) family, as well as importin beta (KPNB1), enabling systematic analysis of AHR binding.

Using co-immunoprecipitation assays, we identified specific importin alpha and beta complexes that interact with AHR. These interactions were confirmed and further validated through analytical size-exclusion chromatography, which allowed us to assess the formation and stability of the complexes in solution. Fluorescence anisotropy measurements revealed that the interactions between AHR and importin alpha proteins occur with high affinity in the nanomolar range, indicating strong and specific binding.

To gain structural insights into these interactions, we initiated structural studies using X-ray crystallography and cryo-electron microscopy (cryo-EM). To date, we have resolved crystal structures of two importin alpha isoforms in complex with a synthetic peptide corresponding to the nuclear localization signal (NLS) of AHR. These structures reveal two distinct interaction sites within the NLS region of AHR that are simultaneously engaged by the importins. Notably, biophysical analysis using biolayer interferometry demonstrated cooperative binding of these two sites, suggesting a concerted mechanism that enhances binding affinity and specificity.

The identification of specific importin partners, coupled with high-resolution structural data, provides a mechanistic framework for understanding how AHR is selectively transported into the nucleus upon ligand activation. Ongoing structural studies aim to complete the picture of AHR-importin complex architecture, which may ultimately inform the development of targeted strategies to modulate AHR activity in physiological and pathological contexts.

#10099

Catching the elusive CeGAL : structural and biochemical insight into the superfamily of transcription factors specific to fungi

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Topics

Microbes

Communication type

Poster

Abstract

Fungi represent a major challenge to both human health and food security, being responsible for recurrent infections (e.g., *Candida albicans*) and devastating agricultural losses (e.g., *Magnaporthe oryzae*). Their remarkable capacity to adapt to selective pressures highlights the need to identify specific targets for the development of novel antifungal strategies.

CeGAL transcription factors (Cysteine-rich GAL4-like) constitute the largest family within the Zn_2C6 superfamily, specific to the fungal kingdom and absent in other eukaryotes. They regulate essential processes such as sporulation, morphogenesis, stress responses, and xenobiotic resistance. Their fungal specificity, combined with their central role in transcriptional regulation, makes them promising therapeutic and biotechnological targets. Despite their abundance (≈350,000 proteins identified), CeGAL factors remain largely uncharacterized at structural and functional levels.

In this study, we characterized two representative members in *Saccharomyces cerevisiae*: PDR8, a regulator of multidrug resistance, and MAL13, an activator of the maltose utilization pathway. Several truncated and MBP-fused constructs were produced in SF9 insect cells. Proteins were purified using combination of HisTrap affinity chromatography, reverse HisTrap, and size exclusion (gel filtration) chromatography. Stabilization approaches (TSA, nanoDSF) enabled the identification of optimal purification buffer conditions, facilitating crystal formation.

These results represent a first step toward understanding the structural and functional properties of CeGAL transcription factors. They lay the groundwork for elucidating the molecular mechanisms governing fungal transcriptional regulation and provide a foundation for exploring CeGAL proteins as selective antifungal targets.

#10209

Molecular basis for the calcium-dependent activation of the ribonuclease EndoU

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Topics

RNA/DNA

Communication type

Oral

Abstract

Ribonucleases (RNases) are ubiquitous enzymes that process or degrade RNA, essential for cellular functions and immune responses. The EndoU-like superfamily includes endoribonucleases conserved across bacteria, eukaryotes, and certain viruses, with an ancient evolutionary link to the ribonuclease A-like superfamily. Both bacterial EndoU and animal RNase A share a similar fold and function independently of cofactors. In contrast, the eukaryotic EndoU catalytic domain requires divalent metal ions for catalysis, possibly due to an N-terminal extension near the catalytic core. In this study, we use biophysical and computational techniques along with in vitro assays to investigate the calcium-dependent activation of human EndoU. We determine the crystal structure of EndoU bound to calcium and find that calcium binding remote from the catalytic triad triggers water-mediated intramolecular signaling and structural changes, activating the enzyme through allostery. Calcium binding involves residues from both the catalytic core and the N-terminal extension, indicating that the N-terminal extension interacts with the catalytic core to modulate activity in response to calcium. Our findings suggest that similar mechanisms may be present across all eukaryotic EndoUs, highlighting a unique evolutionary adaptation that connects endoribonuclease activity to cellular signaling in eukaryotes.

#10219

Structural and functional insights into the nuclear receptor DAF-12 from different parasitic nematodes to develop new anthelmintic molecules

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Topics

Drug Design

Communication type

Oral

Abstract

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Nuclear receptors are ligand-gated transcription factors that regulate essential biological processes, including metabolism, development, and reproduction in metazoans. Due to their lipophilic ligands and ability to modulate multiple genes within the same pathway, they have emerged as attractive targets for the development of therapeutic molecules. Notably, nuclear receptors are present across all categories of helminths, where they contribute not only to development and reproduction but also to survival by enabling adaptation of the worms to various environmental stresses. Among them, DAF 12, the most studied nuclear receptor from nematodes, plays key roles in the development of the infective stage of the parasite and is highly conserved in Caenorhabditis elegans and parasitic nematodes, which made it an interesting drug target. However, its mechanism of action and regulation in parasitic nematodes remains poorly understood, with limited data available on transcriptional coregulators in these organisms. To address this gap, we recently determined the crystal structure of DAF-12 from two parasitic nematodes in complex with mammalian coactivator peptides. Structural analysis, combined with mutagenesis experiments, provided new insights into the receptor's mechanism of action. Based on these structural data and on a comparative genomic analysis of parasitic nematodes, we are now searching for novel coactivators in these organisms. In addition, we identified a peptide of high affinity and specificity for DAF-12 from different parasitic nematodes. A structural study and mutagenesis experiments confirmed the interaction of this peptide on a critical regulatory surface of DAF-12, highlighting its potential as a lead for promising DAF-12 inhibitors development. To further explore this potential, we optimized the sequence and physicochemical properties of this peptide to enhance its functional and pharmacological properties, particularly cellular and nuclear uptake. Preliminary results revealed inhibitory effects of this peptide on DAF-12 activity in cellular experiments and efficient uptake of this peptide by larvae and adult worms, supporting the development of a novel class of DAF-12 targeted anthelmintic agents.

#10245

Multiscale Resolution Structural Studies of Quinone Oxidoreductase: ? -crystallins

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Topics

Other

Communication type

Oral

Abstract

The enzymes of the Quinone Oxidoreductase family (QOR) catalyze quinone or quinonoïd compounds reduction using a two electrons transfer, preventing quinone-induced toxicity. This reaction requires the concomitant oxidation of Nicotinamide cofactors (NAD(P)H). A sub-family of QOR, the ζ-crystallins (ZCr), are prone to catalyze quinone compounds reduction into semiquinone radicals by one electron transfer, with a proven specificity for Nicotinamide Adenine Dinucleotide Phosphate (NADPH)1. Since their discovery, initially in rodent lenses, existence of various ZCr homologous has been enlightened in different organisms, from mammals to plants, yeasts and bacteria. ZCr monomers consists of two super-secondary structures, one of them being a Rossmann Fold allowing Dinucleotide binding like Nicotinamide Cofactors. The overall ZCr tridimensional structure is very similar to the enzymes from the Alcohol Dehydrogenases family2 but without the characteristic zinc binding sites. Previous results showed differences in ZCr oligomeric state according to the organism. While the ZCr from mammal lenses (guinea pig Cavia porcellus & camel Camelus ferus) are homotetramers, the ZCr from the plant Arabidopsis thaliana, the yeast Saccharomyces cerevisiae or the bacteria Escherichia coli are homodimers. The physiological role and the relevance of ZCr tridimensional structure remain unknown. Herein, we propose multiscale resolution structural studies of ZCr and homologous recombinant enzymes from distinct organisms. Structural studies include Native, SDS and IsoElectric Focusing PolyAcrylamide Gel Electrophoresis but also Analytical Size Exclusion Chromatography; Small Angle X-Ray Scattering and ligand induced Fluorescence Quenching. Crystal structures of two mammalians ZCr: C. porcellus and C. ferus have been resolved, being the first resolved tridimensional structures for these two enzymes. Interestingly and in contrast with literature, our results showed that ZCr from C. porcellus is homodimeric. In addition, ZCr from the plant Triphysaria versicolor tend to be monomeric and ZCr from the yeast Candida albicans was found to be homodimeric. Such differences need to be characterized and may have an importance in the catalytic behavior. Because ZCr produces semiquinone radicals, a highly reactive compound that can lead to H2O2 production by reacting with dissolved O2, structural and comparative studies of ZCr are a keystone in the development of biosensors based on H2O2 detection3.

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Keywords: ζ-Crystallin; Quinone Oxidoreductase; Structural Studies; Characterization; One-electron Transfer.

Annexes

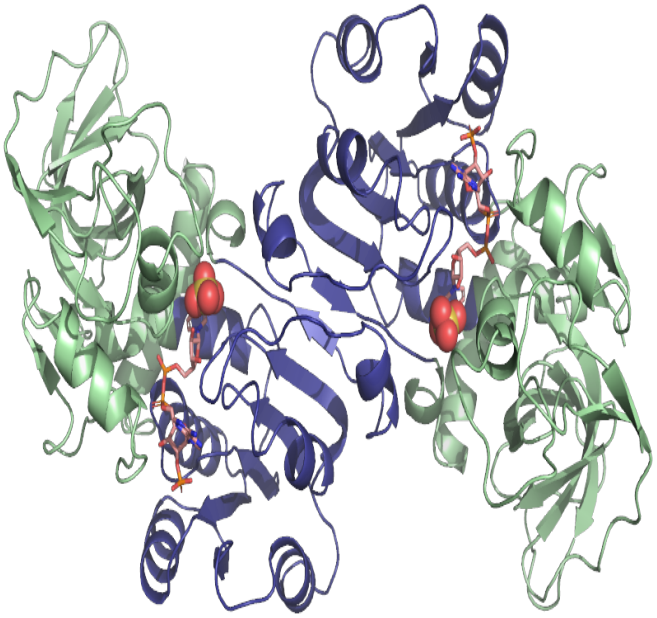

Figure 1: E. coli ZCr-like Structure with NADPH (pdb : 1QOR)

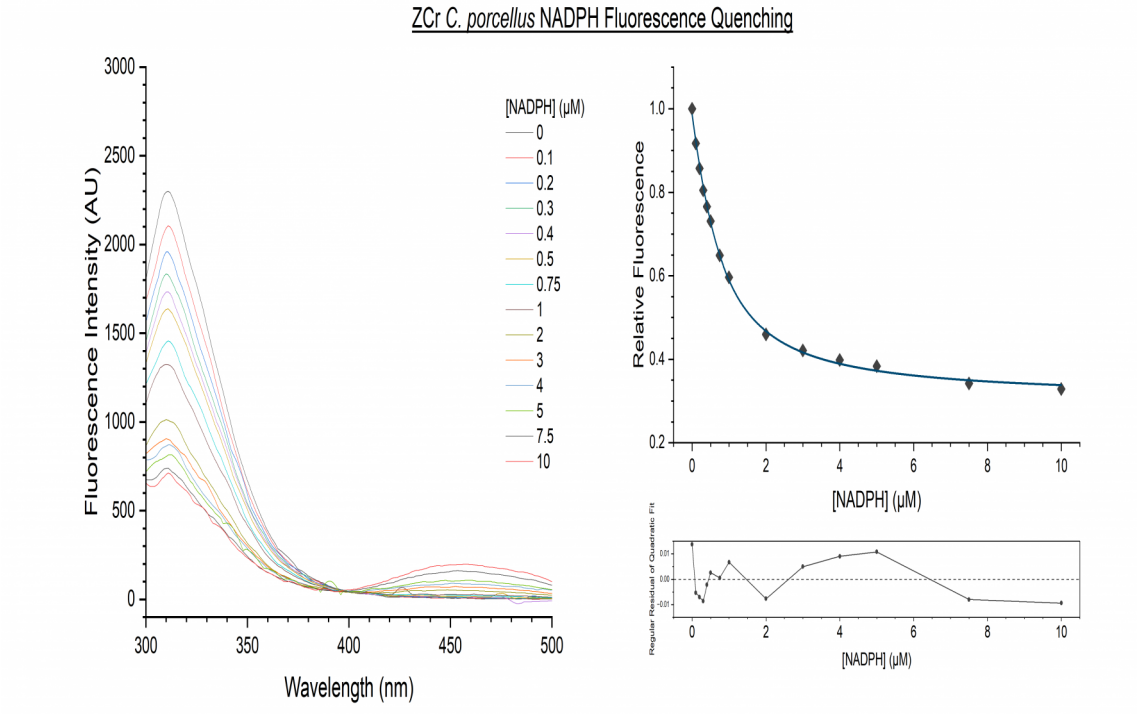

Figure 2: C. porcellus ZCr Aromatic Residue Fluorescence Quenching by NADPH

#10251

Mechanism of Wadjet SMC motor loading on DNA

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Topics

RNA/DNA

Communication type

Oral

Abstract

Structural Maintenance of Chromosome (SMC) complexes are ring-shaped ATPases that play critical roles in chromosome organization, DNA repair, and DNA immunity. SMC complexes utilize ATP-driven opening and closure of their long coiled-coil arms to fold DNA through loop extrusion, processing DNA at rates of hundreds of base pairs per second. Wadjet is an SMC complex specialized in cellular immunity that prevents plasmid transmission in prokaryotes. This defense system comprises a sensor component (JetABC) containing two SMC motor units related to the bacterial condensin MukBEF, and a nuclease effector (JetD) that shares homology with topoisomerases and the meiotic factor Spo11. Previous studies by our group and others have demonstrated that purified type I Wadjet cleaves circular plasmids following comprehensive DNA scanning by the SMC motor. Structural analyses of the cleavage-competent state revealed that both SMC motor units of a single Wadjet complex entrap and deform a small DNA segment, which is subsequently recognized by the JetD nuclease. This observation suggests that Wadjet-mediated loop extrusion involves (topological) DNA entrapment. Here, we investigate the mechanism of Wadjet loading onto circular DNA substrates using cryo-electron microscopy. We have determined the structure of the Wadjet DNA-boarding state, characterized by initial ATP and DNA binding events that position both SMC motor units on a single DNA segment. Using engineered protein complexes and a cryoCysLock strategy, we determined the structure of a DNA-loaded Wadjet complex before loop extrusion begins, revealing how topological DNA entrapment by the SMC motor is achieved.

#10258

Reconstitution and structure-function investigation of regulated cell death signalosomes encapsulated in protocells

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Topics

Other

Communication type

Poster

Abstract

Signalosomes are high-order supramolecular machines involved in immunity, responsible for the activation and execution of regulated cell death. Known as inflammasomes or apoptosomes in mammals and resistosomes in plants, they assemble several protein domains into large suprastructures that act as multi-functional molecular hubs. These supramolecular domain assemblies endow signalosomes with the ability to probe external stimuli, transmit and amplify molecular signals, relocate the machinery to the membrane, and trigger programmed cell death by forming membrane-disrupting pores.

Recently, we have identified similar signalosomes in bacteria and archaea [1]. We hypothesize that the architecture of bacterial signalosomes may be driven by the non-covalent self-assembly of hundreds of amyloid domains into sophisticated supramolecular machinery, analogous to previous studies on fungal signalosomes conducted in our laboratories [2, 3, 4, 5]. We are currently working on a novel type of sequences present in Bacteria that may play a role in an antiphage defense system in Escherichia coli (Bab), with the goal being to better understand the structural and functional intricacies of this protein [6].

Over the past decade, the development of cell-mimicking microcompartments has advanced significantly, exemplified by new cell-mimicking systems developed by our team [7, 8, 9]. These synthetic systems provide a promising alternative for encapsulating complex protein machineries, thereby mimicking the native cell environment. In particular, we recently proposed a compartmentalization methodology to investigate the spatial distribution of a simple intrinsically disordered protein in a crowded environment. However, the application of such methodologies to complex multi-domain machineries has been infrequently proposed [10]. Thus, developing new encapsulation and compartmentalization strategies is necessary to control the proper self-assembly of signalosomes within protocells and to ensure their capacity to execute their biological functions.

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#10259

Structural and Functional Characterization of pioneer factor Oct1 and its Role in Redox Sensing and Chromatin Dynamics.

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Topics

Other

Communication type

Oral

Abstract

Pioneer transcription factors establish regulatory competence by recognising their cognate motifs within nucleosomal DNA and initiating local chromatin opening. Octamer-binding transcription factor 1 (OCT1/POU2F1) is a broadly expressed regulator implicated in development, differentiation, immune signalling, and cellular stress responses. Unlike classical activators that require pre-opened chromatin, OCT1 can access occluded DNA and recruit cofactors to remodel local chromatin architecture. Dysregulation of OCT1 has been linked to tumour progression and therapy resistance, where it promotes survival under oxidative pressure by boosting antioxidant programmes, dampening pro-apoptotic signalling, and favouring glycolysis to limit reactive oxygen species. In parallel, OCT1 participates in the control of type 3 RNA polymerase III promoters (e.g., U6), underscoring its functional breadth. Although pioneer factors have been intensively studied, the mechanistic rules governing nucleosome engagement remain incompletely defined and high-resolution nucleosome-bound structures exist for only a subset of factors. Within the OCT family, robust pioneer activity and nucleosome-bound characterisation have centred on OCT4 (POU5F1), whereas OCT1's capacity to engage nucleosomal DNA and open chromatin remained poorly defined prior to this work, with only indirect evidence from chromatin-based assays.

Here, we present cryo-electron microscopy structures of the human OCT1 DNA-binding POU domain bound to nucleosomes assembled on either the Widom 601 sequence or a genomic human U6 promoter sequence. Across both constructs, the reconstructions reveal three OCT1 molecules simultaneously engaging a single nucleosome. Beyond base-specific readout, the POU domain forms contacts with multiple histone N-terminal tails, including a specific interaction with the histone H4 tail that correlates with local conformational adjustments in the nucleosome. The U6-based structure demonstrates that this engagement mode extends to a physiologically relevant genomic context, mitigating concerns about sequence-encoded bias.

To probe functional consequences for chromatin architecture, we used fluorescence resonance energy transfer (FRET) assays and found that OCT1 can displace linker histone H1, consistent with pioneer-factor-driven exposure of nucleosomal DNA. These results provide a direct mechanistic link between OCT1–nucleosome recognition and the facilitation of chromatin decompaction.

We next examined whether oxidative stress tunes OCT1's DNA binding and subcellular dynamics. In vitro, OCT1–DNA association is strongly inhibited by oxidising conditions and restored by a reducing agent, indicating redox sensitivity of the DNA-binding activity. In cells, immunofluorescence analysis reveals that diamide treatment triggers relocalisation of OCT1 from nucleus to cytoplasm, consistent with a redox-controlled shuttling process. These observations connect the biochemical redox dependence of the POU domain to cell-level regulation of OCT1 localisation.

Together, these data define a structural and biophysical framework for OCT1 pioneer activity. We propose a model in which OCT1's POU domain engages nucleosomal DNA and histone tails to stabilise binding on intact nucleosomes, overcomes H1-mediated compaction to expose DNA, and is gated by the cellular redox state, which modulates both occupancy and subcellular localisation. Given the frequent overexpression of OCT1 in cancer and its role in metabolic and oxidative stress adaptation, our findings highlight the OCT1–redox axis as a potential vulnerability for therapeutic intervention and as a biomarker for patient stratification.

#10260

Quantitative lipid analysis of brain, myelin-enriched and corpus callosum fractions in mice, by multinuclear NMR and mass spectrometry

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Myelin is a key component of the central nervous system. It is a lipid and protein lamellar membrane structure that tightly envelops the axon in a concentric fashion (cochlear structure). Present in the central and peripheral nervous systems of vertebrates, it gives its name to white matter due to its high concentration (80%) of phospholipids, glycosphingolipids and cholesterol. It plays a key role in signal transmission and neuron survival in the central nervous system, and its loss or lack of integrity can lead to a number of neurodegenerative diseases. Although myelin preserves several aspects of biological lipid bilayers, its peculiar membrane organization is still not fully understood. To decipher compositional and dynamics properties of myelin, we proposed a combined NMR and mass spectrometry approach to quantitatively investigate lipid composition. We compared various samples ranting from mice whole brain, extracted myelin-enriched fraction and corpus callosum (bundle of millions of myelinated nerve fibers linking the two hemispheres of the brain) to study their lipids. By comparing control samples, specimens extracted from animals in pathological conditions and in vitro reconstituted lipid membranes, we provided a detailed description of lipid dynamics.

#10264

Tale of the tails: Chasing the evolutionary emergence of histone tails

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Topics

RNA/DNA

Communication type

Oral

Abstract

DNA compaction is a fundamental biological process that allows cells to organize and store their extensive genetic material within the limited volume of the nucleus or nucleoid. In eukaryotes, this compaction is achieved through the assembly of chromatin, a highly dynamic structure in which DNA is wrapped around histone proteins to form nucleosomes. The regulation of chromatin architecture is crucial, as it directly influences accessibility to the genetic code and thereby modulates transcription, replication, and repair. A key layer of this regulation arises from post-translational modifications (PTMs) of histone tails. These chemical modifications, such as methylation, acetylation, and phosphorylation, govern internucleosomal interactions and provide binding platforms for numerous effector proteins that coordinate genome function.

Interestingly, histones are not exclusive to eukaryotes but are also present in Archaea, where they play an important role in genome organization. However, archaeal histones are generally “minimalist” versions of their eukaryotic counterparts, lacking the long and mostly disordered tails that are essential for regulatory processes in eukaryotic chromatin. This is particularly true for the hyperthermophilic archaeon Thermococcus kodakarensis, which encodes two histones, HtkA and HtkB. The absence of tails in these histones raises intriguing questions about how chromatin is regulated in Archaea and how the complexity observed in eukaryotes might have emerged during evolution. Understanding this evolutionary transition is essential for reconstructing the origins of epigenetic regulation.

In this study, we resolved the structure of the nucleosome-like particle formed by HtkA using cryo-electron microscopy (cryo-EM). To probe the evolutionary significance of histone tails, we engineered chimeric archaeal histones by fusing them with eukaryotic tails, generating synthetic models that mimic putative proto-eukaryotic chromatin. By integrating complementary approaches, biophysical assays in vitro, structural analyses by cryo-EM, and functional experiments in vivo in T. kodakarensis, we sought to assess how the acquisition of tails altered chromatin structure, dynamics, and cellular physiology.

#10267

Structural dynamics in signal transduction by dectin-1

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Dendritic cell-associated C-type lectin (dectin-1) is a membrane C-type lectin receptor (CLR) expressed on myeloid cells and involved in immune response to pathogens and damaged host cells. Together with other CLRs, dectin-1 constitutes a potential target for the development of therapeutics able to tune the immune response to infectious pathogens or inflammatory diseases (Mata-Martínez al, Front. Immunol, 2022). It possesses an extracellular carbohydrate recognition domain (CRD), a single-transmembrane domain and an intracellular domain (ICD). The ICD displays a phosphorylable hemITAM motif able to trigger a variety of signaling pathways by recruiting kinase or phosphatase partner proteins. While structural insight into extracellular ligand recognition by dectin-1 has been obtained (Brown et al, Protein Sci, 2007), the mechanisms of signal transduction and intracellular partner protein recruitment remain elusive. Here, we performed SAXS and NMR in solution to investigate the conformational dynamics of the ICD upon phosphorylation and its interaction with the Src homology 2 (SH2) domains of the spleen tyrosine kinase (SYK). Our experiments highlighted the disordered nature of the ICD and revealed that mono-phosphorylation of the hemITAM (pICD) did not alter its conformational state. We observed that a region of the ICD around the hemITAM is animated by more restricted conformational dynamics and displays a propensity to form a-helical structures. Furthermore, the residues within this region undergo large changes in chemical environment upon binding of the pICD with individual N- and C-terminal SH2s, or with the tandem SH2 of SYK (tSH2) with a stoichiometry of $(pICD)_2/(tSH2)_1$. Together with preliminary SAXS and ^{15}N NMR relaxation measurements of pICD – SH2 complexes, these data point to pICD folding upon binding to the signaling partners of dectin-1. These findings enhance our understanding of the conformational dynamics that occur during the first steps of dectin-1 signalling and enable structural studies aiming to decipher the binding selectivity of the ICD in different phosphorylation states for its signalling partners. In longer-term perspectives, we have established a yeast-based expression strategy that enables the production of isotope-labelled full-length dectin-1 and pave the way to integrative studies in membrane-mimetic environments. This approach aims to unravel the molecular determinants of the functional coupling between extracellular ligand binding and intracellular partner protein recruitment.

#10268

Solid-state NMR investigation of the role of the cystine-rich effector CrpA on Aspergillus fumigatus cell wall organization

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Topics

Other

Communication type

Poster

Abstract

Aspergillus fumigatus is a filamentous fungus and a major cause of invasive fungal infections in immunocompromised individuals. The conidial cell wall is the first point of contact with the host and plays a crucial role in protecting the fungus from environmental stress and immune detection. This multilayered structure is composed of an inner rigid scaffold of polysaccharides, including β-1,3-glucan, α-1,3-glucan, and chitin, overlaid by a flexible outer matrix enriched in galactomannan and galactosaminogalactan (GAG). These layers are decorated with surface proteins that contribute to morphogenesis, adherence, and immune modulation.

CrpA is a cysteine-rich conidial surface protein recently identified as a modulator of immune signaling and fungal virulence. However, its potential structural role in cell wall organization has not been investigated. Understanding how such proteins contribute to the spatial arrangement of wall components is crucial for elucidating mechanisms of fungal immune evasion.

To explore this, we used solid-state nuclear magnetic resonance (NMR) spectroscopy, a method capable of revealing the molecular arrangement of insoluble and heterogeneous biological structures without disrupting their native architecture. We used ^{13}C- and ^{15}N-labeled conidia from both the wild-type strain and a CrpA-deficient mutant (ΔCrpA) to examine the detailed composition and spatial distribution of wall polysaccharides.

Comparative NMR analysis revealed changes in the relative abundance and surface accessibility of key polysaccharides, particularly β-1,3-glucan and galactosaminogalactan (GAG). These findings show that CrpA influences both the composition and surface organization of the fungal cell wall. Its absence leads to altered exposure of immunogenic components, which could affect recognition by host immune cells and contribute to reduced virulence.

#10269

Structural study of synaptotagmin-1 and its interaction with lipid membranes by Nuclear Magnetic Resonance

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Topics

Lipids and membrane proteins

Communication type

Indifferent

Abstract

Synaptotagmins (SYTs) are transmembrane proteins essential for calcium-triggered neurotransmitter release, with Synaptotagmin-1 (Syt-1) acting as the primary calcium sensor1. Syt-1 plays a critical role in the rapid and synchronous release of neurotransmitters by interacting with SNARE complexes and facilitating synaptic vesicle fusion. SYTs consist of a luminal N-terminal segment, a single transmembrane domain, and a cytoplasmic domain that binds calcium and phospholipids1.

Recent findings show that the N-terminal and juxta-membrane domains of Syt-1 can interact directly with gangliosides, such as GT1b, in the neuronal membrane, forming a lipid-protein complex essential for toxin binding2,3. These interactions emphasize the importance of lipids in modulating the spatial distribution and function of proteins involved in neurotransmitter release4.

This study aims to investigate the structural and topological interactions of the N-terminal and juxta-membrane domains of Syt-1 with gangliosides and other membrane lipids, using NMR. Our findings will provide insights into the extracellular lipid-protein complexes at nerve terminals, enhancing the understanding of synaptic function and its modulation.

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#10270

Structure and interactions of RocS controlling chromosome segregation in Streptococcus pneumoniae

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Abstract – PhD Project

“Structure and interactions of RocS controlling chromosome segregation in Streptococcus pneumoniae”

Accurate chromosome segregation is a fundamental process for bacterial viability, yet its molecular mechanisms remain poorly understood. In Streptococcus pneumoniae, an opportunistic pathogen responsible for severe invasive diseases, the growing resistance to antibiotics represents a major public health challenge and highlights the urgent need for novel therapeutic targets. In this context, the protein RocS (Regulator of Chromosome Segregation) has recently been identified as a key player in chromosome segregation and its coupling to cell division [1], [2]. RocS combines a DNA-binding domain (N-terminal HtH domain), a predicted coiled-coil segment and a short membrane anchor. Despite its central role, the structure of RocS and the molecular basis of its interactions with DNA and the membrane remain largely unknown. This project aims to elucidate the atomic structure and interaction mechanisms of RocS with both the lipid membrane and DNA by combining magic-angle spinning solid-state NMR spectroscopy with solution NMR and complementary biophysical approaches. The overall objective is to understand how RocS ensures the proper positioning and segregation of chromosomes during the bacterial cell cycle. As a first step, I have started to establish the production and purification of recombinant RocS, paving the way for the preparation of isotopically labeled samples for investigating structures, dynamics and interactions by NMR. In parallel, preliminary work has focused on the study of the membrane anchor of RocS, the key element responsible for membrane binding. Initial data show how RocS can realize the insertion of a short membrane anchor into the specific lipid-protein nanodomains. This work will allow us to explore the interaction dynamics and conformational changes associated with chromosome segregation. In the long term, this project will provide a structural understanding of a central process in the chromosome segregation of a pathogen listed by the WHO as a “medium-priority antibiotic-resistant bacterium” and could open perspectives for the development of antibacterial strategies targeting the division and propagation of S. pneumoniae.

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Thesis supervised by Dr. Birgit Habenstein

#10272

Structures of functional oligomers of protein NS5A from highly replicating strains of hepatitis C virus (HCV)

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Topics

Virology

Communication type

Poster

Abstract

Hepatitis C virus (HCV) is a major global health issue. Its genome encodes a single polyprotein cleaved by cellular and viral enzymes into three structural proteins (core, E1, E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B). A hyperreplicative strain of HCV was recently identified in a liver transplant patient (Heuss et al., PLoS Pathog 2022). This genotype 1b strain, referred to as GLT1 (German Liver Transplant 1) causes dramatic rearrangements of host cell membranes. This is associated with mutations of NS5A in a region (aa 237-342, Fig1A), thus termed Replication Enhancing Domain (ReED). In this region, substitution P237L was found as necessary and sufficient to confer the hyperreplicative phenotype in a consensus genotype 1b (gt1b) background and increase the dimerization of NS5A in huh7_lunet cells (P. Rothhaar & V. Lohmann, personal communication). Cyclophilin A (CypA) is a host peptidyl prolyl cis-trans isomerase, that is recruited by HCV to interact with NS5A, particularly with proline-rich motifs within the D2 domain (Hanoulle et al., Journal of Biological Chemistry 2009).

NS5A is a membrane-associated protein, essential for both replication and assembly of the hepatitis C virus (HCV). It also plays a critical role in membrane remodeling in HCV infected cells. NS5A consists of three domains (AH-D1, D2, D3), connected by short low complexity sequences (LCS), and is anchored to the membrane via its N-terminal amphipathic helix (AH).

NS5A is known to self-associate through its domain D1. However, structural predictions using AlphaFold suggest that an additional dimerization interface may be present within the ReED region in D2. To characterize these novel dimerization properties, we analyzed peptides of various lengths (short and extended, Fig 1B, C) in ReED, by using solution NMR, fluorescence spectroscopy and Isothermal Titration Calorimetry. The peptides were produced in E. coli labelled and unlabelled. 15N and 13C-labeled peptides were produced to perform NMR assignment.

CypA binding is known to stabilize conformers of D2. Given that CypA engages multiple sites in D2, we hypothesized that it might interact with the putative dimerization region. This interaction could stabilize the peptide and potentially promote its dimerization. We used NMR and Isothermal Titration Calorimetry studies to investigate the binding of CypA on the peptides of interest.

Our NMR data indicate the presence of an α-helix within the region predicted to be helical by AlphaFold. NMR titration experiments revealed chemical shift changes indicative of interaction between the extended peptides and CypA, which were further validated by ITC. Together, these complementary approaches confirm the binding of CypA to the extended peptide. Additional experiments will be required to determine how this interaction may stabilize the peptide conformation and potentially influence its dimerization.
As a next step, we will focus on a longer peptide construct, comparing versions with and without the P237 residue, to assess how this position modulates dimerization and how it may be linked to the hyper-replicative phenotype.

Annexes

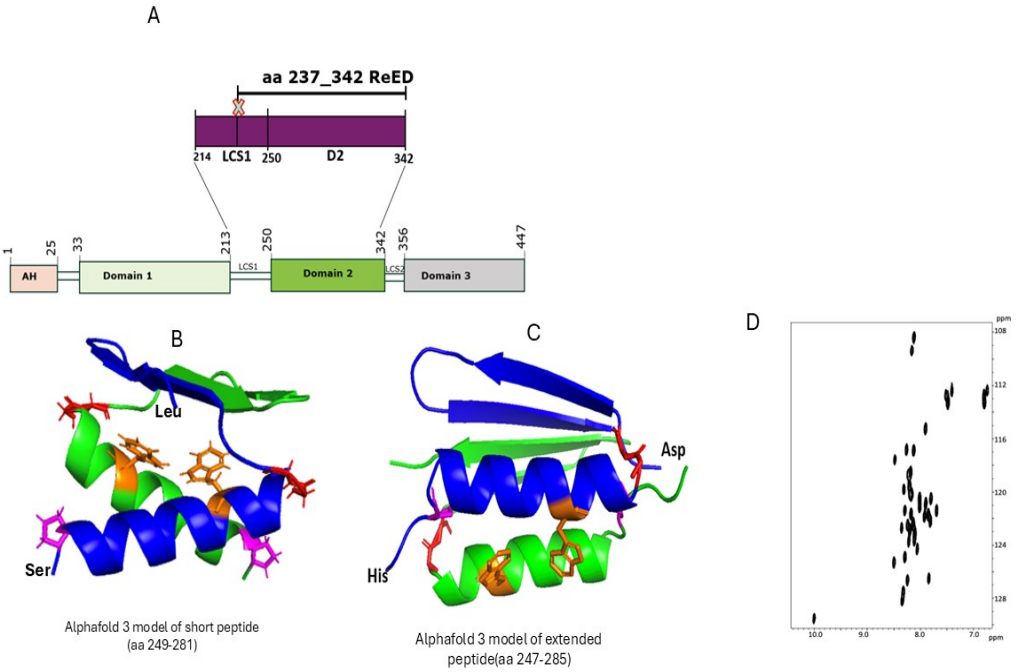

Figure 1: Fig 1 A: Domain organization of NS5A, expanded view of LCS1_D2 region containing ReED, the cross represents position 237 that is the single most important for the highly replicating phenotype in a gt1b context. B-C) AlphaFold 3 models of the dimeriza

#10274

Driving drug repurposing forward through biophysical approaches in the RePo-SUDOE project

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Topics

Drug Design

Communication type

Poster

Abstract

Developing a new drug from scratch is a time-consuming and costly process, often taking more than 10 years and over one billion euros, with a high risk of failure along the way. Drug repurposing, offers an efficient alternative by identifying new uses for existing or previously unsuccessful drugs. Since these compounds already have safety, pharmacokinetic, and manufacturing data available, this strategy can considerably reduce development time, costs and risk. The RePo-SUDOE project, an Interreg EU co-funded initiative, aims to position the SUDOE region (Spain, Portugal, and France) as a European reference in drug repurposing. To this end, it focuses on building an open-access database of therapeutic targets and applying both computational and experimental methods to assess the biological relevance of promising drug candidates.

As a proof-of-concept model, we focused on cancer, where the need for faster and more efficient therapeutic strategies is particularly urgent. To develop robust workflows for drug repurposing, we selected the Hippo pathway effector YAP-TEAD complex. This transcriptional regulator is frequently dysregulated in cancer, driving uncontrolled cell growth and therapy resistance, making it a biologically relevant and therapeutically promising system to evaluate our repurposing strategies.

The YAP-TEAD complex contains three distinct interaction surfaces, presenting promising targets for compounds that can disrupt its oncogenic activity. We reviewed the literature and selected three molecules previously reported to interfere with its interaction. Based on our selection, we are developing robust workflows using biophysical methods such as Surface Plasmon Resonance (SPR) and Bio-Layer Interferometry (BLI) to accurately and reproducibly characterize drug-target interactions. The workflows will support drug repurposing efforts in the SUDOE region and are designed to be adaptable to other therapeutic targets in the future.

#10275

NMR SPECTROSCOPY TO STUDY PROTEIN ASSEMBLIES ON THE BACTERIAL MEMBRANE

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Keywords: Antimicrobial resistance, cell division, Z-ring, ZipA protein, NMR Spectroscopy

Antimicrobial resistance in bacteria is an escalating public health threat, making it crucial to identify new therapeutic targets [1]. Understanding the atomic mechanisms behind protein-membrane interactions in bacteria offers a promising strategy to discover novel drug targets. Our research focuses on the ZipA protein, which plays a vital role in bacterial cell division [2], controlling the initial molecular steps during set-up of the division site in certain bacterial pathogens such as Escherichia coli.

Using both solution and solid-state Nuclear Magnetic Resonance (NMR) spectroscopy, we investigate the molecular structure of ZipA in a membrane-mimicking environment. By targeting the protein structure in a native-like setting, this study aims to uncover insights into the structures and the interactions with the membrane at the atomic level, providing crucial information for the development of new antimicrobial agents.

#10276

Cell-free production of challenging therapeutic targets to accelerate drug repurposing in RePo-SUDOE project

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Topics

Drug Design

Communication type

Poster

Abstract

Obtaining sufficient quantities of high-quality protein targets is often a major bottleneck in the development of new therapeutic compounds, slowing down both drug discovery and repurposing efforts. Conventional expression systems can struggle with proteins that are difficult to fold, unstable or toxic. Cell-free protein production offers a rapid and flexible alternative, enabling the expression of challenging targets in a controlled environment while overcoming many limitations associated with traditional systems. This approach provides the quantity and quality of protein necessary for reliable evaluation of candidate compounds, thus supporting a more efficient drug repurposing workflow.

Within the RePo-SUDOE project, we apply cell-free systems to produce high-quality proteins for downstream applications in our pipeline. As a proof-of-concept, we focused on the YAP-TEAD complex, a key regulator in the Hippo signaling pathway that is frequently dysregulated in cancer. The small TEAD-binding domain of YAP is difficult to produce in Escherichia coli, yielding only limited amounts of protein. To overcome this limitation, we use cell-free methods to obtain sufficient quantities of functional protein for detailed biophysical analysis, including Surface Plasmon Resonance and Bio-Layer Interferometry, to study its interaction with TEAD in the presence of inhibitors targeting this protein-protein interaction.

Ultimately, we aim to establish a robust cell-free workflow that is adaptable to a wide range of therapeutic targets difficult to obtain by other techniques, providing a versatile platform to improve characterization of candidate compounds across cancer and diverse diseases.

#10279

Study of the plasticity of the tripartite complex PCNA-NucS-DNA in Pyrococcus abyssi

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Topics

Other

Communication type

Poster

Abstract

DNA mismatch occurs during multiple processes such as: replication, homologous recombination, chemical agents... These mismatches play a crucial role in generating genetic diversity, facilitating adaptation and specifying traits in microbial populations. The MMR (mismatch repair) pathway serves as a safeguard against the transmission of these mutations. The usual and already known pathway is the canonical, ATP dependent pathway, except that bioinformatic analysis failed to identify the latter in many archaeal and bacterial genomes, such as Pyrococcus abyssi and Streptomyces ambofaciens respectively. This allowed us to identify a new MMR pathway, named noncanonical, and independent of ATP. PCNA/ β-clamp (archaeal/bacterial) and endonuclease NucS are the key proteins in this pathway. This alternative pathway opens up new questions around the structure and the activity of these two proteins with the DNA. In archaea, specifically Pyrococcus abyssi, PCNA-NucS shows high affinity (Kd= 15nM). In order to further understand this pathway, we intend to study the structure of the tripartite complex: MMDNA-PCNA-NucS. In parallel, bacterial NucS structure is not discovered yet, so we aim to get the structure of it in order to compare it with the archaeal NucS, using crystallography and SAXS. Lastly, Thermococcus kodakarensis NucS showed affinity towards DNA carrying GT, GG, TT mismatches and low affinity towards AC mismatches. As expected, using fluorescence anisotropy, PabNucS displayed the same affinity profile, with the order of affinity being: GG>GT>TT and showing very low affinity toward AC and control (perfectly matched) sequences.

#10281

Biochemical and structural analyses of arylsulfate-sulfotransferases (ASSTs)

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Topics

Other

Communication type

Oral

Abstract

Sulfotransferases (STs) are a diverse superfamily of enzymes that catalyze the transfer of sulfate to hydroxyl or amine groups. This superfamily of sulfotransferases can be divided into two large families: PAPS-dependent sulfotransferases (ST) and PAPS-independent sulfotransferases or arylsulfate sulfotransferases (ASST). PAPS-dependent sulfotransferases were discovered first, mainly present in eukaryotic organisms, and are the most extensively studied in human model organism. In comparison, arylsulfate sulfotransferases, which are mainly found in bacteria, remain underexplored (1–4). To enhance our knowledge on ASSTs and building upon phylogenetic analyses that divides ASSTs into 22 clades, our work aims to elucidate the sequence–structure–function relationships underlying substrate specificity within the family of ASST by using biochemical and structural studies.

Guided by bioinformatics, we investigated 18 ASSTs (25 constructs) covering diverse clades, of which 16 ASSTs were successfully purified. Structural studies resulted in the determination of crystal structures for two representatives: the first one from Desulfitobacterium hafniense from clade 6 (DH_ASST_6) and the second from Salmonella typhimurium from clade 5 (ST_ASST_5), in both apo and complexed form with donor or acceptor substrates. The study also includes activity assays on artificial acceptors that provide functional insights, supporting the hypothesis that different clades are specific of different substrates. Notably, the ST_ASST_5 crystal structures, allowed to identify a strictly conserved substrate-binding site present in all sequences belonging to clade 5, strongly supporting clade-specific substrate recognition mechanisms, at least for some of the clades.

Together, these results offer new structural and biochemical insight into ASSTs activities and lay the foundation for understanding their clade-dependent substrate specificity.

Legend

Right: complete 3D structure

Left: pNPS trapped in the catalytic site between two aromatic residues (Phe-174 and Tyr-556) next to the active site.

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Annexes

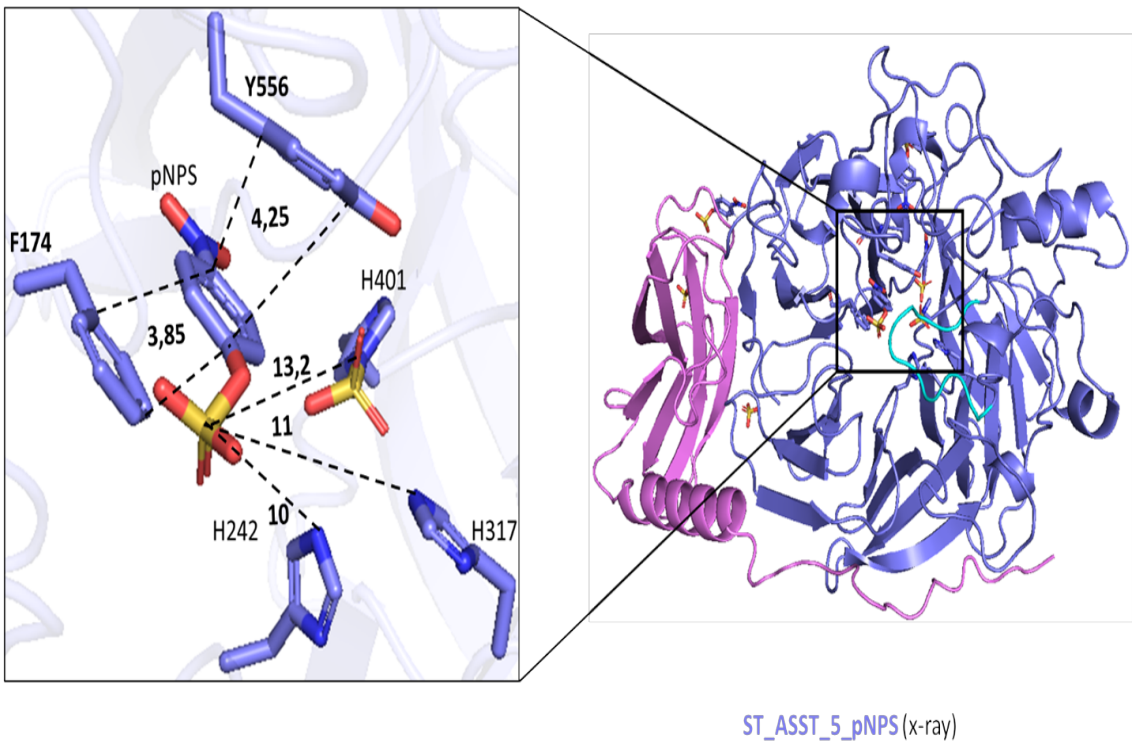

Figure 1

#10284

Activity modulation in anaerobic ribonucleotide reductases (aRNR)

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Topics

RNA/DNA

Communication type

Oral

Abstract

Organisms store their genetic information in DNA, thus making deoxyribonucleotides (dNTPs) essential components of the cellular replication and proliferation machinery. The enzyme ribonucleotide reductase (RNR) catalyses the reaction that provides new DNA building blocks.

There are three different classes of RNRs (I, II, III) different in reactivity towards oxygen, cofactor requirement, and quaternary structure, even within subgroups of each class. A fine-tuned allosteric regulation occurs within the enzyme to control the amount of dNTPs available in the cell. Allosteric effectors ATP or dATP bind to an N-terminal regulatory domain called ATP-cone that acts as an on/off switch of the enzyme. Although RNRs are widely studied, their mechanisms of regulation are largely unknown.

The details of dATP-inhibition have so far only been described for class I RNRs, in which case large oligomeric complexes are formed, excluding formation of the enzymatically active dimer-of-dimers between the two RNR subunits. In contrast, class II and III RNRs consist of a single subunit, in most cases forming a homodimeric enzymatically active complex, suggesting that the mechanism of dATP-inhibition is different from that of class I.

Recently, we published a comprehensive study including seven high-resolution single-particle cryo-electron microscopy (cryo-EM) reconstructions of the bacteria Prevotella copri NrdD that suggest a novel mechanism whereby dATP binding to the so-called “ATP-cone” domain results in inactivation by disordering of the critical C-terminal region containing the glycyl radical. Taken together with biochemical and biophysical data, we could show that the enzyme is inactivated not by prevention of initial formation of the radical but by prevention of its transfer to the substrate.

Annexes

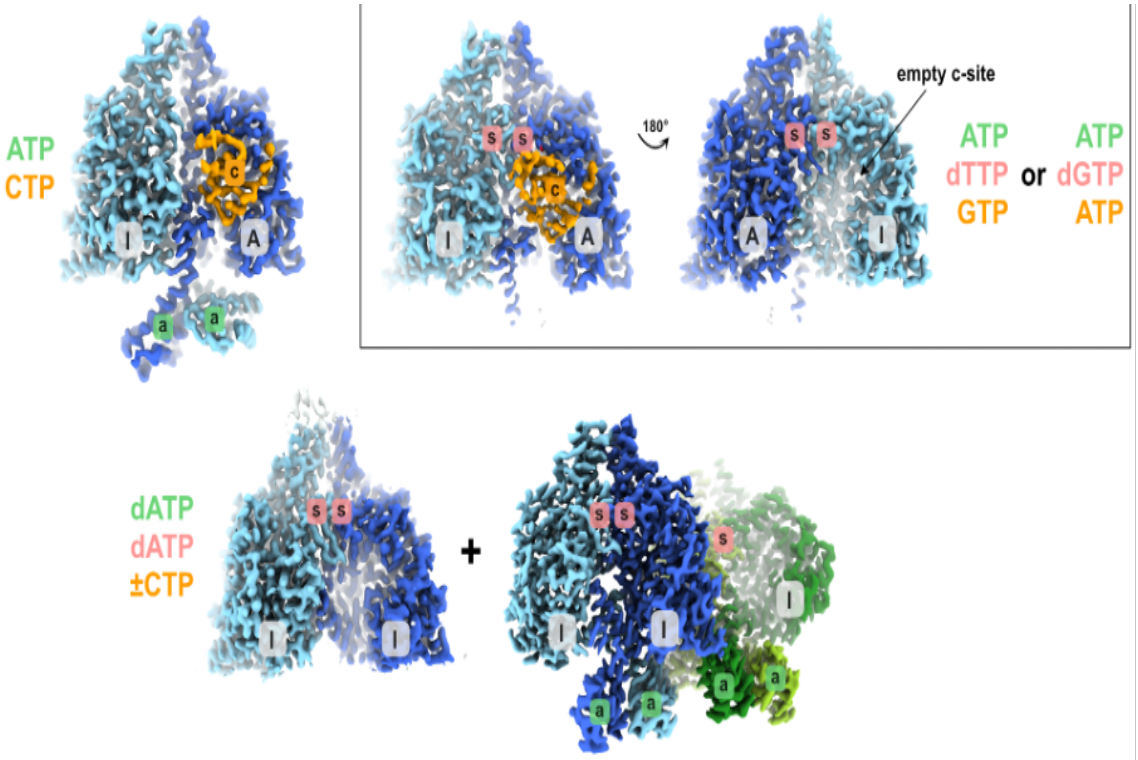

Figure 1

#10291

The disordered region of human Poly(A) Polymerase controls mRNA polyadenylation site selection

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Topics

RNA/DNA

Communication type

Oral

Abstract

Human polyadenosine polymerase alpha (PAPα) is crucial for 3'end pre-mRNA maturation, adding a polyadenosine (poly-A) tail to preferred distal sites to ensure mRNA stability, facilitate nuclear export, and regulate gene expression. Despite its critical role in mRNA maturation, several aspects of PAPα function remain poorly understood, notably the function of its 250-amino acid intrinsically disordered region (IDR).

Here, we use artificial intelligence (AI)-based predictions (AlphaPulldown), including a segmental pull-down strategy to identify an interaction between the IDR of PAPα and the Cleavage Factor CFIm25, a key regulator of alternative polyadenylation. Using nuclear magnetic resonance (NMR) spectroscopy, we characterize the structural properties of the full-length IDR, and we determine the precise CFIm25 binding site within the IDR using titrations, R_{1rho} relaxation and chemical exchange saturation transfer (CEST) experiments. Exploiting NMR-guided crystallization screening, we obtain the crystal structure of the CFIm25-PAPα complex at 1.4 Å resolution. Guided by this structure, we determine CFIm25 mutants that disrupt its interaction with PAPα, providing a basis for assessing the functional impact of their interaction in human cells. Our cellular experiments reveal that the IDR of PAPα acts as a molecular ruler, linking CFIm25 recognition of the upstream UGUA pre-mRNA motif to selection of the downstream distal polyadenylation site by the catalytic core of PAPα. Disruption of this interaction leads to a shift toward proximal polyadenylation site usage across thousands of genes, a hallmark of several cancer types. Our data uncover an unanticipated telescripting mechanism by which PAPα controls the selection of polyadenylation sites, providing fundamental insight into mRNA biogenesis and its misregulation in disease. More broadly, our work highlights the wider functional significance of IDRs in cellular regulation.

#10292

Mechanism of translational miscoding and inhibition by the odilorhabdin class of context-dependent peptide antibiotics

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Topics

Other

Communication type

Oral

Abstract

Odilorhabdins are peptide antibiotics produced by the nematode-symbiont bacteria *Photorhabdus* and *Xenorhabdus* sp. They target the prokaryotic 30S ribosomal subunit and inhibit bacterial translation in a sequence- and concentration-dependent manner. Previous studies have revealed that these antibiotics cause miscoding at low concentrations and additionally inhibit translocation at higher concentrations. Here, we investigate the molecular mechanism of translation inhibition by NOSO-502, the first clinical candidate from the odilorhabdin class aimed at treating *Enterobacteriaceae*-induced catheter-associated urinary tract infections (cUTIs), using biochemical and structural approaches. Using high-throughput inverse toeprinting coupled with next-generation sequencing (iTP-Seq), we show that NOSO-502 preferentially stalls translation at specific sequence contexts in a concentration-dependent manner. To gain further insight into the mechanism of action of the drug, we determined ensemble cryo-EM structures of ribosomes stalled by NOSO-502 during translation of a representative arrest motif. The structures include various conformations of the bacterial 70S ribosome with tRNAs in different stages of elongation, including a fully-accommodated non-cognate tRNA in the ribosomal A site. Overall, these structures reveal how the antibiotic perturbs decoding center dynamics and tRNA discrimination, and suggest that increased miscoding induced by NOSO-502 may ultimately lead to impaired tRNA–mRNA translocation.

#10294

Cytosolic human NAD kinase, a promising target for cancer treatment

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Topics

Cancer

Communication type

Poster

Abstract

Human nicotinamide adenine dinucleotide kinase (HsNADK1) is an enzyme that catalyzes the phosphorylation of nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide phosphate (NADP). NAD and NADP are two essential cofactors playing an important role in many metabolisms, protein regulation and oxidative stress response.

Recently, the NADK activity was demonstrated to be crucial for cancer cell proliferation upon phosphorylation by the protein-kinase Akt (1). Since then, NADK activity was shown to be upregulated in metastatic breast cancers (2). Therefore, inhibiting cytosolic HsNADK1 is a promising anti-cancer strategy. However, this important therapeutic target is still orphan with no potent inhibitor described yet.

For a long while, just one crystal structure of HsNADK1 in its apo-form, was known (PDB3PFN). Last year, we have solved the structure of the full-length HsNADK by cryo-EM at a resolution of 3.2 Å in absence of ligand, which shows interesting rearrangements compared to the crystal apo-form (3). However, studies with its natural ligands, NAD and NADP, appeared difficult due to significant aggregation. This led us to design a shortened variant, named HsNADK_esv with lower enzymatic activity but improved solubility. The cryoEM structure of this variant in complex with NAD could be solved at 2.3 Å (3). Nevertheless, the pace of ligand screening using cryoEM is still limited and we would like to obtain crystals suitable for X-ray diffraction. We have engaged in co-crystallization trials with this construct.

In addition, we are interested in understanding the functioning and the regulation of this enzyme. Solving its structure with NADP at atomic resolution will be important. We wish also to understand the impact of point mutation observed in various cancers. Therefore, we recently introduced a proline-to-serine mutation at position 200 (P200S), that is observed in melanoma and corresponds to a position near the hinge connecting the two domains of this enzyme. Crystallogenesis is being undertaken on these two constructs, and we recently solved a first structure of HsNADK short8 P200S at 4 Å resolution. However, further refinement is required to achieve higher resolution and visualize ligand interactions.

These structural data should help us in optimizing inhibitor design in the near future.

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Keywords: HsNADK1, metastasis, structure, point mutation, inhibitor

#10297

Structure-based design of the very first PXR PROTAC, a route to PXR antagonism and control of chemoresistance

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Topics

Drug Design

Communication type

Oral

Abstract

The nuclear receptor PXR (pregnane X receptor) is traditionally considered a xenobiotic receptor, and as such, it regulates the transcription of metabolizing enzymes and drug transporters, thereby facilitating the removal of xenobiotics from the body. Thanks to its large and versatile ligand-binding pocket (LBP), PXR can accommodate a wide range of ligands with very different structures, sizes, and chemical properties. Consequently, PXR plays a role in the early stages of drug metabolism, which can result in adverse effects, such as drug-drug interactions, resistance to anticancer therapies, or the accumulation of toxic metabolites. To prevent these undesirable interactions and enhance the efficacy of treatments, pharmacological inhibitors of PXR are necessary. Based on structural data [1, 2], we developed a novel approach using the Proteolysis Targeting Chimera (PROTAC) technology to inhibit PXR. We designed a bifunctional chimeric ligand, JMV7048*, which binds to PXR with high affinity and can recruit the proteolytic machinery to induce its degradation. The rational development of this molecule was made possible through the gradual conversion of a homemade agonist and by relying on the structures of PXR complexes with intermediate molecules. This process once again demonstrated the extraordinary adaptability of the LBP of this receptor, and the structures will be presented here. From a functional perspective, JMV7048 specifically degrades PXR in colon carcinoma, hepatoma, and pancreatic cancer cell lines, but not in primary cultures of human hepatocytes. Importantly, this molecule reduced PXR protein expression in colon cancer stem cells and sensitized them to chemotherapy, significantly delaying cancer relapse in vivo. The development of these PXR PROTAC molecules will therefore enable new therapeutic strategies to be considered in combination with other anti-cancer drugs to improve the efficacy of conventional treatments.

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*Patent WO2022243365 - BIFUNCTIONAL PROTAC-TYPE COMPOUNDS TARGETING PXR

#10298

AI-assisted Design of Nanobodies and Mini-Proteins for High-Affinity Therapeutic Binders

Liza AMMAR-KHODJA (CNRS)

Topics

Peptides

Communication type

Poster

Abstract

AI-assisted Design of Nanobodies and Mini-Proteins for High-Affinity Therapeutic Binders

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The ANDES project (Artificial-Intelligence-based Methods for Nanobody and Mini-protein Design) aims to establish a computational experimental framework to accelerate the engineering of binders with high specificity and affinity. Nanobodies, single-domain antibody fragments derived from camelids, are particularly attractive due to their small size, stability, and solubility. Building on this concept of compact binders, mini-proteins small artificial proteins generated by de novo design (as pioneered by groups such as David Baker's) push the boundaries even further by offering versatile scaffolds that are smaller than nanobodies yet capable of reaching comparable or superior affinities. Together, nanobodies and mini-proteins represent complementary approaches to develop next-generation therapeutic and diagnostic molecules. Traditional discovery pipelines, however, remain time- and resource-intensive.

The project is developed in close collaboration with teams specializing in artificial intelligence and computational protein design. Building on advances such as the EffieDes hybrid AI framework, novel algorithms integrating backbone flexibility, conformational sampling of antigen-binding loops, and binding specificity optimization are explored. These methods have been applied to SARS-CoV-2 as a model system. First, the MR17 nanobody was computationally redesigned to restore recognition of the emerging RBD variant XBB.1.16. Several mutant candidates were generated in silico and tested experimentally by biolayer interferometry (BLI), confirming binding restoration and providing leads for crystallographic structural validation.

In parallel, a large panel of de novo designed mini-proteins was produced and screened against the Delta variant RBD. These compact synthetic binders combine stability and high affinity despite their reduced size. Remarkably, one candidate displayed picomolar binding affinity (KD in the low pM range). Crystallization trials have been initiated to resolve its structure and benchmark the accuracy of AI-based predictions.

Altogether, ANDES demonstrates the potential of combining AI-based design (developed by collaborators) with rigorous experimental validation. The project provides a foundation for a fast, adaptable, and scalable platform for therapeutic binder discovery, with broad applications in infectious disease preparedness and biomedical innovation.

#10299

Functional and structural studies of the HIV-1 uncoating and integration steps in condensate phases environment.

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Topics

Virology

Communication type

Oral

Abstract

The pre-integration steps of the HIV-1 viral cycle are prime targets for the latest therapeutic innovations. They involve the viral integrase (IN), a multifunctional protein with conformational flexibility (high inter domain flexibility and containing intrinsically disordered regions). These types of proteins have been characterized by their ability to induce liquid-liquid phase separations (LLPS) and to form molecular biomolecular condensates also called membrane-less organelles (MLOs), enabling them to evolve in a restricted microenvironment. It has been shown that MLOs are involved in the nuclear uncoating of the HIV capsid. We investigated here if such complexes can form LLPS in vitro and if IN enzymatic activities were affected by this LLPS environment. For these studies, we developed an original setup to produce proteins, complexes in mammalian cells (Drillien et al., PlosOne, 2022). HIV-1 integrase, hLEDGF, IN/LEDGF complex, hCPSF5 and hCPSF6 are produced in mammalian cells. HIV-1 capsid monomers, hexamers and capsid rods have been produced in E. coli. The viral capsid core is purified from non-replicative virus. Functional assays for IN have been setup both in standard and in the LLPS context, showing strong effects of the LLPS environment on the IN enzymatic activities. We found that the LLPS formed by IN-LEDGF/p75 functional complexes modulate the in vitro IN activities. While the 3'-processing of viral DNA ends was drastically reduced inside LLPS, viral DNA strand transfer was strongly enhanced. These two catalytic IN activities appear thus tightly regulated by the environment encountered by intasomes (Batisse et al., JBC, 2024). To go further in theses studies, we conduct structural investigations of the HIV-1 capsid core and rods – CPSF5/6 complexes by cryo-electron tomography (CryoET) and IN/LEDGF nucleosome complex by single particles cryoEM. A preliminary structure at ~10 Å resolution has been obtained by cryoET sub-tomogram averaging for the capsid core and rods in complex with hCPSF5/6 (Figure 1).

Annexes

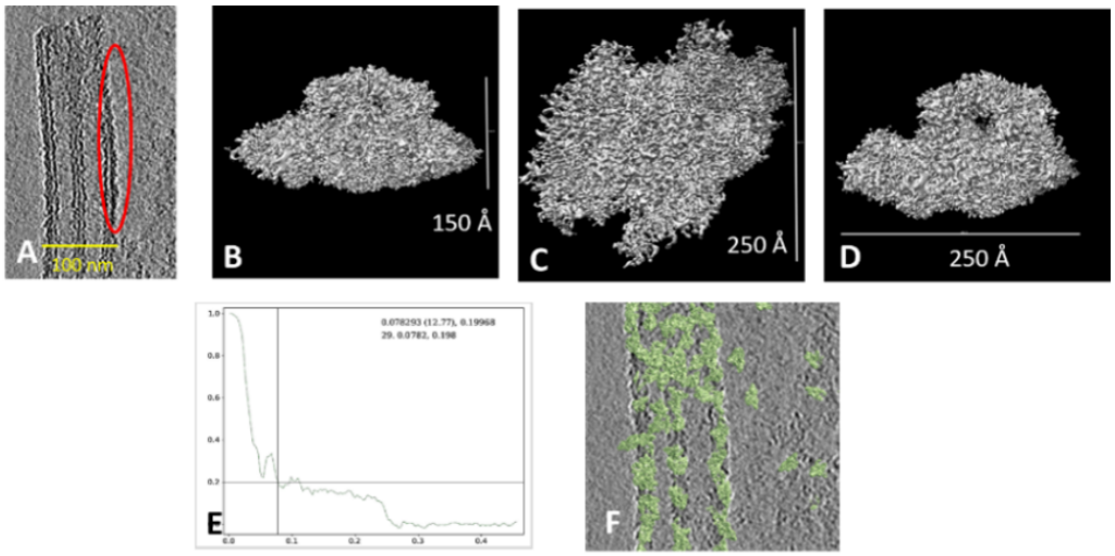

Figure 1: **A**: Tomogram of CA hyper stable rods in complex with CPSF5/6, **B, C, D**: Sub-tomogram averaging: Refinement with 1819 3D particles, resolution ~13 Å, **E**: Fourier Shell Correlation (FSC at a threshold of 0.2: 12,77 Å), **F**: sub-tomograms superimposed on tomograms of capsid rods.

Figure 1

#10300

Structural and functional study of the HIV-1 Integrase-LEDGF complex

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Topics

Virology

Communication type

Indifferent

Abstract

HIV integration into the host genome is a crucial step in the viral replication cycle, yet the structural and molecular mechanisms governing this process remain incompletely understood. HIV integrase (IN), in cooperation with the host factor lens epithelium-derived growth factor (LEDGF), catalyze preferential viral DNA insertion into actively transcribed regions, where nucleosomes serve as key targets. However, the precise interactions between IN–LEDGF and chromatin requires further elucidation.

To address this, we investigated the structural basis of IN–LEDGF–nucleosome interactions using integrative structural and biophysical approaches. Our work focused on optimizing the production and purification of the IN/LEDGF complex in a eukaryotic expression system [1] to ensure proper folding and stability under physiological conditions. Through buffer optimization, particularly by adjusting ionic strength, we enhanced complex stability, thereby improving conditions for structural and functional studies.

Bio-layer interferometry (BLI) experiments confirmed a strong interaction between IN/LEDGF and nucleosomes, with dissociation constant (Kd) values in the nanomolar range. These findings support the hypothesis that LEDGF acts as a tether for chromatin-targeted integration. Additionally, optimized conditions improved the efficiency of functional assays, including 3′-processing and strand transfer reactions. We are currently optimizing grid preparation for IN/LEDGF–nucleosome samples to understand the structural basis of HIV-1 integration. Initial attempts using detergents or streptavidin-coated grids yielded only separate IN–LEDGF complexes and nucleosomes without detectable interactions. We therefore adopted the strategy previously used in BLI experiments, where strong binding between the complex and nucleosomes was observed at nanomolar affinity.

In parallel with these studies, we are investigating the IN/LEDGF complex alone by optimizing vitrification conditions, since its structure has not yet been determined. We have currently achieved an overall resolution of 3.3 Å (Figure 1) and are processing the data to resolve different conformational states of the complex, with the aim of building the first high-resolution structure of the IN–LEDGF complex.

These findings provide insights into how HIV exploits chromatin for efficient integration and suggest broader implications for retroviral replication. Future efforts will focus on refining high-resolution structures, incorporating additional host and viral cofactors, and exploring potential inhibitors targeting the IN–LEDGF interface. By advancing our understanding of HIV integration, this work may lead to therapeutic strategies aimed at modulating integration site selection and limiting viral persistence.

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#10303

Human myeloperoxidase: from fundamental to applications

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Topics

Other

Communication type

Oral

Abstract

Mono- and multi-species biofilms are responsible for a wide range of chronic infections that are difficult to eradicate and significantly impact both quality of life and mortality. As a result, there is growing interest in developing broad-spectrum antimicrobial strategies that act directly at the infection site without promoting microbial resistance.
Myeloperoxidase (MPO, structure in Figure 1), a member of the heme-dependent peroxidase superfamily, not only oxidizes classical peroxidase substrates into radical intermediates but also uniquely catalyzes the formation of hypochlorous acid (HOCl) from hydrogen peroxide and chloride ions (Eq. 1), a reaction responsible for the antimicrobial activity of bleach:
Eq. 1: $H_2O_2 + Cl^- + H_3O^+ \rightarrow HOCl + 2H_2O$
Commercial human MPO is typically isolated from blood or produced recombinantly in mammalian cells or E. coli. Alternative approaches have explored bacterial homologs of MPO [1–2].
This work focuses on:
i) the production of human MPO in *Pichia pastoris*;
ii) one-step purification of the active recombinant enzyme;
iii) its integration into coupled enzymatic systems or chimeric enzymes (with or without a linker) that generate HOCl from glucose;
iv) its immobilization on silicon surface models; and
v) its biophysical and structural characterization using atomic force microscopy and tryptophan fluorescence quenching.
Results include insights into enzyme production, purification, enzymatic activity, surface immobilization, and structural analysis. Comparative studies were performed between coupled systems and chimeric enzymes linking two catalytic domains. The functional role of the linker in these constructs is also discussed.

KEYWORDS: cationic myeloperoxidase, coupled enzymatic system, chimera, bifunctional enzyme, anionic glucose oxidase, QCM adsorption.

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Annexes

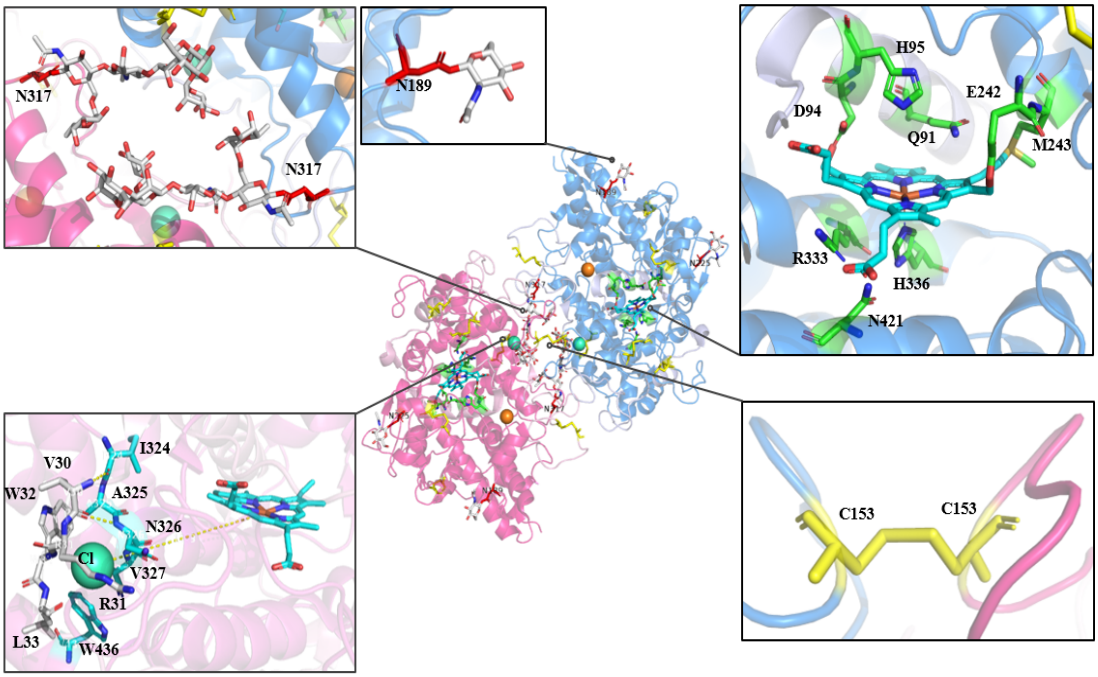

Figure 1: hMPO structure (from pdb code 1CXP)

Figure 1

#10305

The auxiliary protein gp12 of bacteriophage SPP1, a model of prokaryotic collagens

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Topics

Virology

Communication type

Poster

Abstract

Collagens are trimeric fibrillar proteins that are characterized by a strict repetition of a Gly-X-Y motif. While such motifs are well-characterized in eukaryotes, their structural and functional roles in prokaryotes remain largely unexplored. One such protein is the small auxiliary protein gp12 of bacteriophage SPP1 that is present at the capsid surface.

We determined the crystal structure of gp12, revealing a tripartite architecture composed of an unstructured N-terminal region, a collagen triple helix, and a C-terminal coiled-coil. We also determined an icosahedral reconstruction of the mature SPP1 capsid, that showed how gp12 interacts with the major capsid protein gp13. This interaction involves salt bridges between basic residues in the N-terminal domain of gp12 and acidic residues of gp13. Comparative genomics further identified gp12-like proteins across diverse phages, suggesting that collagen-like domains may be more widespread in prokaryotic viruses than previously recognized.

The gp12 collagen appears to be stabilized by interchain interaction involving polar residues. Further systematic bioinformatic study on collagens shows that these polar residues are over represented in collagens from all three domains, suggesting their important role in collagen stability.

#10306

Structural insights into tRNA sulfuration by [4Fe-4S]-dependent modification enzymes

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Topics

RNA/DNA

Communication type

Oral

Abstract

The ubiquitous nucleoside modification 5-aminomethyl-2-thiouridine (xnm5s2U) at uridine 34 in the anticodon loop of transfer RNAs is crucial for genetic translation. Biochemical and spectroscopic studies have previously shown that several bacterial MnmA enzymes catalyze sulfuration of U34-tRNA using a [4Fe-4S] cluster, like their archaeal/eukaryotic counterpart. The cluster is coordinated by three residues belonging to a conserved D/CXXC + C motif, three cysteines in thermophilic bacteria and one aspartate and two cysteines in Escherichia coli. We report here spectroscopic and catalytic assays that confirm that the [4Fe-4S] cluster is needed for tRNA thiolation in the case of two Gram-positive bacterial MnmAs, together with X-ray data that enabled to visualize the coordination state of the [4Fe-4S] cluster. We observed a [4Fe-5Se] cluster in the crystal structure at 2.37 Å resolution of the Asp-to-Cys mutant of Streptococcus pneumoniae MnmA, after cluster reconstitution using selenide, supporting the existence of a [4Fe-5S] cluster as a catalytic intermediate. Co-expression of Bacillus subtilis MnmA (BsMnmA) with its specific cysteine desulfurase YrvO led to the formation of a complex that was efficient in tRNA thiolation using cysteine as the sulfur source only after cluster reconstitution. The structure of the BsMnmA/YrvO/tRNA complex by cryoelectron microscopy at 3.44 Å resolution shows that the catalytic cysteine of YrvO is located in close proximity to a conserved cysteine of MnmA, outside the cluster binding site, suggesting that the persulfide on YrvO's cysteine could attack the MnmA cysteine to form a disulfide bond and liberate hydrosulfide. The latter could then be channeled to the [4Fe-4S] cluster and serve as the sulfur source to modify the uridine targeted by MnmA.

Annexes

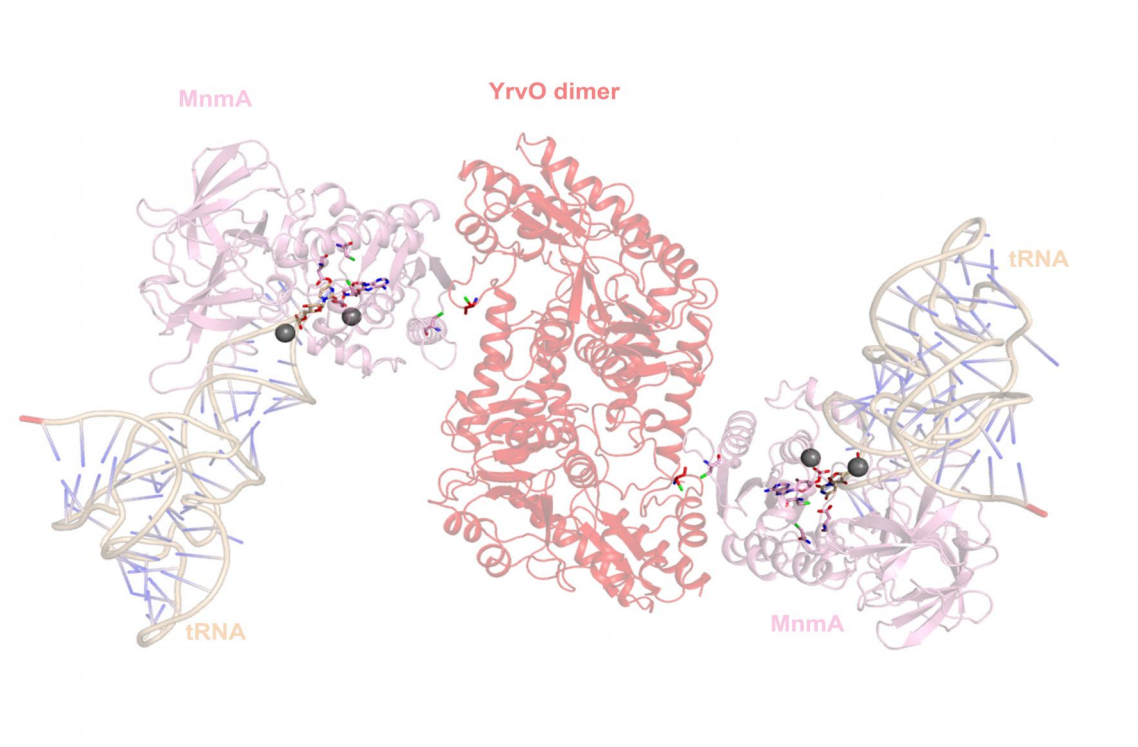

Figure 1

#10308

Restoring Azole Sensitivity in Drug-Resistant Candida Through Cdr1 Pump Inhibition

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Topics

Lipids and membrane proteins

Communication type

Indifferent

Abstract

The fungal kingdom comprises over 5 million species, including yeasts, molds, and mushrooms. Fungal infections affect around a billion people globally, with 3 million developing invasive forms that cause approximately 1.5 million deaths annually. Candida albicans is the most common fungal pathogen, but drug-resistant species like Candida glabrata are emerging as a major concern. These resistant strains cause invasive candidiasis with high mortality rates, ranging between 40% and 60% (Lass-Flörl et al, 2023; Fidel et al, 1999).

Azole antifungals, currently the most widely used class, act by targeting lanosterol 14α-demethylase (ERG11), a key enzyme in fungal cell membrane synthesis. However, their extensive use has led to the emergence of resistance mechanisms, particularly through overexpression of the Cdr1 efflux pump in Candida species, which expels azoles from the fungal cell and reduces drug efficacy (Aoyama et al, 1984; Sgherri et al, 2014).

As a promising approach to overcome antifungal resistance, we aim to inhibit Cdr1-mediated efflux by screening in vitro derivatives of the commercial azoles posaconazole and itraconazole, with the goal of blocking the Cdr1 efflux pump in both clinical Candida isolates and laboratory Saccharomyces cerevisiae strains. Toxicity tests were first performed on strains overexpressing Cdr1 in order to identify the most active compounds. Among the twenty molecules tested, one exhibited the desired effect, with greater toxicity than posaconazole. The effect on Cdr1 was then assessed by measuring its ATPase activity in membrane fractions. These tests showed that the most toxic compound indeed inhibits Cdr1. Unexpectedly, these tests also showed an activation of Cdr1's ATPase activity by the substrates it transports— something never before observed in PDR-type (pleiotropic drug resistance) family proteins, to which Cdr1 belongs. These findings highlight the diversity of interaction mechanisms with Cdr1 and led to identify the first azole antifungal that effectively inhibits its primary target while also blocking Cdr1, the efflux pump meant to eliminate it.

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#10309

Optimizing overexpression of the antifungal transporter Cdr1 in yeast membranes enables reliable measurement of its inhibition for drug screening

Mme Nour SAMROUTH (PHD student)

Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Fungi infect around one billion of people, with 3 million suffering from invasive infections, leading to around 1.5 million deaths annually [2]. C. glabrata [3] is naturally multi-drug resistant and cause invasive candidiasis with high mortality rates, ranging between 40% and 60% [5] . Azole antifungals are the most used class, however, their widespread use and long-term application have led to the emergence of resistance. C. glabrata resists azole therapies by overexpressing Cdr1, a membrane transporter that expels azole out of the cell, thus decreasing their intracellular concentration.

Our team resolved the structure of Cdr1 in several conditions, and notably in complex with Itraconazole [7], a molecule used in therapy. Based on these structural insights, a series of itraconazole derivatives were designed to enhance antifungal activity. For this purpose, I optimized the Cdr1 expression levels to obtain enriched membrane fractions that would improve screening reliability. I modified several factors, including extended preculture incubation, culture incubation temperature, the optical density at the time of induction, as well as the duration and conditions of protein expression. These optimizations significantly increased the expression of Cdr1 in the membrane. I measured the sensitivity of Cdr1 in membrane to oligomycin and FK506, reference inhibitors, and evaluated twenty molecules, which lead to the identification of one with greater toxicity than posaconazole, representing a potential future therapeutic agent.

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#10312

Investigate the dynamics and molecular mechanism of a bacterial molecular motor involved in Iron Transport

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Topics

Lipids and membrane proteins

Communication type

Indifferent

Abstract

The Molecular motor formed by the ExbB-ExbD protein complex is located in the inner membrane of bacteria. This molecular motor energizes the import of scarce nutrients (metals, vitamins, sugars) through the bacterial envelope via a specific outer membrane transporter. The energy is generated by the proton-motive force or the proton gradient of the inner membrane, and is transferred to the outer membrane transporter via a third protein TonB/HasB [1]. The transferred energy is used to open a channel through the transporter allowing the nutrient entry.

The complex formed by ExbB-ExbD and a TonB paralog HasB is part of the heme acquisition system (Has). To import heme, which is the major source of iron for bacteria within mammalian hosts, this system transfers energy through interdependent motions between ExbB-ExbD, HasB and transporter.

Recent studies from our laboratory have identified the interface between the periplasmic domain of the molecular motor and HasB, which overlaps with the region recognizing bacterial peptidoglycan. In addition, it has been shown that this interaction is transient [2]. However, the structural and mechanistic details of the ExbB–ExbD–HasB complex, its energizing mechanism, and the role of peptidoglycan remain unknown.

Understanding these processes necessitate a scrupulous molecular and atomic-level investigation of the interactions between various proteins of the Has system and peptidoglycan, as well as their dynamics. To do so, we will use a combination of experimental approaches (NMR) and molecular dynamics (MD) simulations in a model envelope and in the presence of peptidoglycan.

As a first step, we study by MD and NMR the sequential binding of HasB and peptidoglycan to the molecular motor. Experiments revealed that ExbD can directly interact with HasB and peptidoglycan. ExbD binds HasB even in the presence of peptidoglycan. In addition, steric hindrance involving disordered regions may play a critical role in the ExbD-binder interaction. To address this, we are currently simulating the whole protein-membrane system, including ExbD, HasB, and the lipid membrane.

The resulting knowledge could shed light on the regulation of bacterial nutrient uptake and the development of novel antibacterial strategies.

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2. M. Zinke et al., Ton motor conformational switch and peptidoglycan role in bacterial nutrient uptake. Nature Communications 15, 331 (2024).

#10316

Structural basis for human mitochondrial tRNA maturation

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Topics

RNA/DNA

Communication type

Oral

Abstract

The human mitochondrial genome is transcribed into two RNAs, containing mRNAs, rRNAs and tRNAs, all dedicated to produce essential proteins of the respiratory chain. The precise excision of tRNAs by the mitochondrial endor- ibonucleases (mt-RNase), P and Z, releases all RNA species from the two RNA transcripts. The tRNAs then undergo 3′-CCA addition. In metazoan mitochondria, RNase P is a multi-enzyme assembly that comprises the endoribonuclease PRORP and a tRNA methyltransferase subcomplex. The requirement for this tRNA methyltransferase subcomplex for mt-RNase P cleavage activity, as well as the mechanisms of pre-tRNA 3′-cleavage and 3′-CCA addition, are still poorly understood. Here, we report cryo-EM structures that visualise four steps of mitochondrial tRNA maturation: 5′ and 3′ tRNA-end processing, methylation and 3′-CCA addition, and explain the defined sequential order of the tRNA processing steps. The methyltransferase subcomplex recognises the pre-tRNA in a distinct mode that can support tRNA-end processing and 3′-CCA addition, likely resulting from an evolutionary adaptation of mitochondrial tRNA maturation complexes to the structurally-fragile mitochondrial tRNAs. This subcomplex can also ensure a tRNA-folding quality-control checkpoint before the sequential docking of the maturation enzymes. Altogether, our study provides detailed molecular insight into RNA-transcript processing and tRNA maturation in human mitochondria.

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#10317

Post-translational acylation drives folding and cytotoxicity of the CyaA bacterial toxin

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Topics

Lipids and membrane proteins

Communication type

Oral

Abstract

The adenylate cyclase (CyaA) toxin is a major virulence factor secreted by Bordetella pertussis, the causative agent of whooping cough. CyaA is produced as an inactive precursor, proCyaA, which is activated by the acylation of two lysine residues within the bacterium. Post-translational acylation of CyaA is well recognized as a molecular modification critically required for the efficient intoxication of target cells. However, how acylation shapes CyaA conformation in its active functional state remains unclear. Once acylated and secreted, CyaA invades innate immune cells and disrupts their phagocytic functions. High-resolution structural characterization of CyaA has been hindered by its size, multi-domain organization, flexibility, and propensity to aggregate. Here, we overcome these challenges and present the first structural ensembles of both non-acylated and acylated CyaA in solution by combining experimental data with integrative modeling. Coarse-grained molecular dynamics simulations reveal that acylation is critical for stabilizing the native fold and favorably orienting CyaA on the target membrane. Overall, our findings show how post-translational acylation drives native folding and provide mechanistic insights into the early steps of host cell intoxication.

#10324

Transitions of NHEJ short-complexes revealed by cryo-EM

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Topics

RNA/DNA

Communication type

Oral

Abstract

We present new cryo-electron microscopy (cryo-EM) structures and corresponding atomic models of a key step in DNA repair by non-homologous end joining (NHEJ), captured just before ligation. We reconstituted the complete Saccharomyces cerevisiae NHEJ machinery and assembled a super-complex with a blunt-ended double-strand break (DSB) substrate modified to prevent ligation. In addition, we determined the structure of a complex with cohesive ends containing four base pairs of microhomology. Together, these structures provide a foundation for modelling the dynamic transitions between different NHEJ substrates during the final stages of DNA end joining.

The reconstructed complexes reveal a symmetric architecture consisting of two Ku70/80 heterodimers bound to the DNA ends, flanking two Lif1 (XRCC4) homodimers bridged by a central Nej1 (XLF) homodimer. The synaptic complex also contains two Dnl4 (Ligase IV) molecules, with well-resolved DNA-binding (DBD) and N-terminal (NTD) domains. The overall architecture mirrors the organization observed in the human NHEJ system, suggesting evolutionary conservation between yeast and humans, while also revealing distinct structural features that suggest the existence of a yeast-specific mechanism governing the final stages of DNA repair by NHEJ.

#10329

FBDD of a bacterial kinase inhibitor capable of increasing antibiotic sensitivity of multidrug-resistant clinical isolates

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Topics

Drug Design

Communication type

Poster

Abstract

According to the World Health Organization (WHO), antibiotic resistance is a growing global health problem. One way to combat this problem is to block the mechanisms used by bacteria to resist antibiotics. Some bacteria can inactivate a group of antibiotics called aminoglycosides by using enzymes such as aminoglycoside phosphotransferases (APHs). These enzymes inactivate antibiotics by phosphorylating them. In our study, we used a fragment-based drug design approach to identify and synthesize a new compound capable of inhibiting these enzymes. This compound increases the antibiotic sensitivity of multidrug-resistant clinical isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. It works by competing with ATP, the phosphate donor. Although the results are promising, further optimization is necessary to enhance the compound's effectiveness against bacteria, particularly by increasing its bioavailability.

This work was supported by the ANR (SIAM ANR-19-AMRB-0001), the Association Vaincre la Mucoviscidose (RF20220503015), the Association Grégory Lemarchal, the Canadian Institutes for Health Research Foundation Grant (FDN148463), the German Federal Ministry of Education and Research (BMBF, SIAM-APH, No. 16GW0235, grant number: 01KI2126B), the Fonds France-Canada pour la Recherche, and Mitacs.

#10331

Drug binding on the Human multidrug transporter ABCG2and mutants from natural polymorphism; comparaison between purified protein in FC14 and DDM

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

The human ABC transporter ABCG2 plays a key role in cancer drug resistance by mediating the efflux of anticancer agents, thereby reducing their intracellular accumulation and contributing to multidrug resistance. A detailed understanding of these mechanisms is essential for the development of more effective anticancer therapies.

I expressed wild-type (WT) ABCG2 in E. coli, along with two naturally occurring polymorphism variants: V12M, associated with gastric cancer, and Q141K, linked to gout. While ABCG2 can be extracted from the E. coli membrane using fos-choline-14 (FC14), it is not directly extractable with DDM. Given that the protein purifies well in FC14, initial drug-binding assays were performed in this detergent.

However, due to FC14's reputation as an "SDS in disguise", which may compromise the functional integrity of membrane proteins, we developed a strategy to reconstitute ABCG2 into liposomes from FC14-purified samples, followed by re-solubilization in DDM. This allowed us to reas

#10333

Development of Inhibitors for the Histone Chaperone ASF1

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Topics

Drug Design

Communication type

Poster

Abstract

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 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.

#10343

Structural Basis for Synthase Activation in the E. coli Type II cellulose secretion system

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Bacteria have evolved sophisticated nanomachines for the biogenesis of biofilm matrix components leading to cooperative multicellularity. Cellulosic polymers constitute a prevalent class of matrix exopolysaccharides that rely on cyclic diguanylate (c-di-GMP)-dependent cellulose synthases.

The latter's core is structurally conserved across kingdoms but polymer structure and modifications depend on the ensemble of synthase modules and accessory subunits, thus defining several types of bacterial cellulose secretion (Bcs) systems.

In *E. coli* and other enterobacteria, a multicomponent Bcs macrocomplex, encompassing the inner membrane and cytosolic subunits (BcsRQABEFG), and an outer membrane porin (BcsC) secure the biogenesis of phosphoethanolamine (pEtN)-modified

cellulose. Resolution-limited studies have proposed different Bcs macrocomplex stoichiometries and its assembly and regulation have remained elusive.

Using cryo-EM, we visualize the molecular mechanisms of BcsA N-terminal domain-dependent recruitment and stabilization of a trimeric BcsG pEtN-transferase for polymer modification and the BcsF-dependent recruitment of an otherwise

cytosolic BcsE2R2Q2 regulatory complex that stabilizes the BcsA glycosyl transferase and c-di-GMP-sensing PilZ domains into a pre-activated state.

Importantly, we demonstrate that BcsE, a secondary c-di-GMP sensor, remains c-di-GMP-bound, retains its BcsRQ partners onto the synthase and thus mantains the overall pre-activated conformation even in the absence of direct c-di-GMP-synthase complexation, effectively lowering the threshold for dinucleotide-dependent activation.

Such activation-by-proxy mechanism would allow Bcs secretion system activation even in the absence of dramatic intracellular c-di-GMP increase and is reminiscent of the dinucleotide-dependent activation in other synthase-dependent polysaccharide secretion systems, where c-di-GMP sensing is carried out by key co-polymerase subunits.

#10347

Experimental and computational study of the protein-membrane interaction of the proposed N-terminal HEV ORF1 domain MetY

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Topics

Virology

Communication type

Poster

Abstract

Positive-sense single-stranded RNA ((+)RNA) viruses create replication vesicles, in which they store a template copy of their genome, protecting it from detection from the host and allowing them to safely replicate their genetic material. The formation of replication vesicles is mediated by the assembly of a membrane-bound protein complex that deforms the membrane into what is commonly known as a membrane pore, although in viruses that form single-membrane vesicles it does not actually cross the bilayer. Cryo-electron microscopy (CryoEM) and tomography (CryoET) studies on four (+)RNA viruses (an insect virus, a coronavirus, a norovirus, and Chikungunya virus (CHIKV)) have shown that the “pore-forming” protein exists as a membrane-associated dodecamer and is responsible for the recruitment of other viral proteins to the replication complex. In the Alphavirus-like superfamily of (+)RNA viruses, which includes CHIKV, this function is executed by the RNA capping enzyme.

There is very little knowledge about the life cycle and function of the non-structural proteins of Hepatitis E Virus (HEV), a member of the Alphavirus-like superfamily. Nevertheless, there are convincing indications that the methyltransferase N-terminal domain (MetY) of the HEV replication polyprotein (ORF1) would perform this function. AlphaFold models of this domain are most structurally homologous to nsP1, CHIKV’s “pore-forming” protein. Further analysis of this modelling strongly suggests that it dodecamerises into a ring, with suspected membrane-interacting regions on its outer edge. Additionally, the N-terminal region of ORF1 has been proven experimentally to be directed towards membranes in insect and human host cells.

For my PhD, I analyse this hypothesis using two distinct but complementary approaches. On one hand, I simulate the molecular dynamics of different oligomeric states of MetY and their interactions with biological membranes. The simulation of dimers demonstrated a strong membrane anchorage through two α helices, which have previously been described as a membrane director and a crucial region for viral replication. These helices correspond to the suspected membrane-interacting regions identified in the molecular modelling and are highly suitable membrane anchors for oligomerisation. On the other hand, I perform biochemical assays to assess the membrane interaction of MetY. Cell-free protein synthesis based on wheat germ extract permits not only the production and purification of detergent-solubilised MetY for membrane interaction assays, but also its production directly in the presence of membranes. I identify membrane-bound MetY through sucrose density gradients. To further assess MetY association to membranes and to study its oligomerisation, I will perform simulations of more complex systems, CryoEM, other biochemical approaches, as well as cellular localisation and viral replication assays involving helix mutations.

#10356

Présentation de la ligne de lumière Cryo-EM POLARIS

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M Heddy SOUFARI (CEA)

Topics

Other

Communication type

Poster

Abstract

Présentation de la ligne de lumière Cryo-EM POLARIS récemment ouverte au Synchrotron SOLEIL.

#10358

Overcoming the bacterial antibiotic resistances using a macrolide-antimicrobial peptide chimera

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Topics

Drug Design

Communication type

Indifferent

Abstract

In 2019, antimicrobial resistance (AMR) was associated with an estimated 4.95 million deaths worldwide. Projections also show that antimicrobial resistance could kill up to 10 million people by 2050 while also threatening animal health and the agricultural sector. To address this growing health crisis, the World Health Organization has designated a group of pathogens, known as “ESKAPE(E)” (E.faecium, S.aureus, K.pneumoniae, A.baumannii, P.aeruginosa, Enterobacter spp., and E.coli), as critical targets for the development of new antibiotics. In this context, we are developing a strategy to avoid the emergence and dissemination of resistance by designing conjugates in which macrolides are covalently linked to small proline-rich antimicrobial peptides (PrAMPs). Macrolides are bacteriostatic antibiotics commonly used against Gram-positive pathogens, while PrAMPs, naturally produced by arthropods and mammals, exhibit bactericidal activity against Gram-negative bacteria. Both of these classes of molecules interact with the peptide exit tunnel (PET) of the ribosome. Our group had previously shown that, unexpectedly, both macrolides and PrAMPs can coexist within the PET. By conjugating a macrolide with a PrAMP, we aim to combine their activities, potentially overcoming macrolide resistance, extending the antimicrobial spectrum of macrolides and limiting the emergence of resistances. Biochemical experiments, such as in vitro translation, were conducted to select the best candidates to drive the thermodynamic assays on ITC. Taking into account the results of both chemical and physical parts, we will resolve the structures of the ribosomes in complex with the compounds by cryo-electron microscopy. These approaches open promising avenues for the development of next-generation antibiotics.

#10359

Structural snapshots of D-loop formation in Archaea revealed by Cryo-EM

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Topics

RNA/DNA

Communication type

Oral

Abstract

Genome maintenance in bacteria, archaea and eukaryotes critically depends on homologous recombination (HR), which enables the exchange of strands between homologous DNA molecules. During HR, a recombinase binds to ssDNA and forms a right-handed helical nucleoprotein filament, known as a presynaptic filament, which then searches dsDNA for homology. Upon encountering a homologous sequence, the presynaptic filament pairs with the complementarity strand, resulting in the displacement of the non-complementarity strand form the duplex to generate a displacement loop (D-loop) and promote DNA strand exchange. In all forms of life, DNA recombinases share a conserved ATPase domain for ATP binding. ATP is required for the self-association of protomers into an active nucleoprotein filament. In bacteria, HR relies on RecA while in eukaryotes it relies on Rad51/Dmc1. While Rad51 is the universal recombinase in eukaryotes, meiosis also requires Dmc1, which enables mismatch-tolerant inter-homologue recombination. In archaeal cells, the reaction of HR is performed by RadA. To uncover the specific features of archaeal recombinases, we determined the cryo-EM structures of RadA at distinct stages of the homologous recombination reaction: in its apo form, pre-synaptic, synaptic, and post-synaptic states. These structures highlight the evolutionary relationships between archaeal and eukaryotic recombinases. At the molecular level, they also reveal the mechanism of homologous recombination, showing how conserved domains and flexible DNA-binding loops orchestrate the successive steps of strand exchange and pairing. Together, these insights provide a detailed structural framework for understanding how archaeal recombinases mediate homologous recombination with both efficiency and specificity.

#10363

Studying Photoswitching in a Novel Photochromic Flavoenzyme-Inhibitor Complex by Kinetic X-ray Crystallography

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Topics

Other

Communication type

Poster

Abstract

Photochromic proteins (PPs) are light-sensing systems that reversibly switch between two states with distinct absorption spectra and associated structural changes upon light excitation. PPs have attracted significant interest as powerful tools in the life sciences, particularly for applications in super-resolution fluorescence microscopy and optogenetics (1). However, many of the known systems suffer from low photoswitching quantum yields (QY), and limited red- light absorption, important to increase penetration within biological media.

Monomeric sarcosine oxidase (MSOX) is a bacterial flavoenzyme that naturally catalyzes the oxidative demethylation of sarcosine to glycine in a light-independent manner, using its flavin cofactor as a redox intermediate. MSOX can bind the substrate-analogue methylthioacetate (MTA) to form a charge-transfer (CT) complex via close interactions between its flavin cofactor and MTA. The formation of this complex leads to the appearance of a new absorption band in the red region of the spectrum, and it has been recently demonstrated that photoexcitation of the CT complex induces ultrafast (~300 fs) photoswitching with a near-unity quantum yield (QY). It has been also demonstrated the ability to recover thermally within the nanosecond timescale, making it a promising candidate for fast, efficient red-light-activated switching (2).

Our project aims to uncover the structural dynamics underpinning this ultrafast photoinduced CT switching by using various kinetic crystallography approaches. As a first step, we combined in situ illumination, cryo macromolecular crystallography and in crystallo microspectrophotometry at the ESRF FIP2 beamline. We have obtained high resolution (1.7 Å) X-ray crystal structures of the MSOX–MTA complex without and with prior illumination using a LED (530 nm) at 80 K, informing about light-induced structural changes. Upon photon absorption, the CT complex dissociates as monitored by spectroscopy, and the MTA ligand undergoes a conformational rearrangement as monitored by crystallography.

Our next step will be to carry out time-resolved serial femtosecond crystallography (TR- SFX) experiments at XFEL facilities. These experiments aim at capturing photoswitching intermediates at room temperature, thereby tracking the conformational dynamics of the MTA ligand and its interaction with the flavin with femtosecond-to-nanosecond temporal resolution. We have successfully established a protocol to form large quantities of MSOX-MTA microcrystals that diffract to 2 Å resolution at the ESRF microfocus beamline 23-2. The TR- SFX approach will allow us following photoswitching from the Franck-Condon excited state of the CT complex to the dissociated product and back to the resting state.

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#10365

Structural and functional study of the membrane protein Patched

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Some cancerous tumors can resist chemotherapy due to the activity of the Hedgehog signaling pathway receptor, Patched, which can expel chemotherapeutic agents and thus contribute to multidrug resistance. Patched is the receptor of the hedgehog morphogen involved in the development and stem cell homeostasis. it is overexpressed in many cancers. Studies conducted by collaborators at IPMC (Sophia Antipolis) demonstrated that Patched can eject chemotherapy drugs, such as doxorubicin, from cancer cells, thereby helping tumors survive treatment. They also identified inhibitors, such as Paniceine A hydroquinone (PAH), which enhance the effectiveness of chemotherapy.

Our project aims to purify and characterize the Patched protein, both biochemically and structurally. Using cryo-electron microscopy (cryo-EM), the goal is to locate the ligand binding site. SEC-MALS analysis confirmed that Patched is monomeric, and co-purification experiments showed its interaction with Sonic Hedgehog (confirming Patched structuring). Functional studies between purified Patched and PAH revealed an affinity with a defined Kd, supporting our collaborators' in vivo results. Patched has been reconstituted into liposomes to study its efflux function in the presence of the PAH ligand and substrate. Doxorubicin fluorescence will be used to monitor changes in proton transport activity, thereby providing insights into the role of Patched in efflux and resistance mechanisms.

#10369

Structural insights into cross-seeding mechanisms toward amyloid fibrillation

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Topics

Other

Communication type

Oral

Abstract

Protein aggregation into amyloid fibrils is the hallmark of many diseases including Alzheimer's disease (AD), Parkinson's disease (PD) and type 2 diabetes (T2D). Amyloid fibrils feature a universal "cross-β" core structure composed of arrays of β-sheets and can be formed by a variety of peptides or proteins without any evident sequence similarity. Each amyloid-based disease is typically characterized by the aggregation of specific proteins: amyloid-beta protein (Aβ) in AD, α-synuclein in PD and the humanislet amyloid polypeptide (hIAPP) in T2D. Interestingly, cross-seeding, where fibrils from one protein accelerate the aggregation of another, has been observed in multiple disease contexts [1]. As a hormone, hIAPP circulates in the bloodstream and can reach different targets, including the brain. Postmortem studies have found hIAPP co-localized with Aβ and α-syn in brain tissues from AD and PD patients [2]. To investigate this cross-talk, we explored in vitro how different amyloid proteins influence hIAPP aggregation and morphology. Thioflavin T fluorescence kinetics, circular dichroism (CD) spectroscopy, atomic force microscopy (AFM), and transmission electron microscopy (TEM) were combined to assess the impact of various amyloid seeds, including Aβ40, Aβ42, α-synuclein, and a synthetic mutant peptide (H18R-hIAPP), which forms fibrils more slowly than wild-type hIAPP. Our results indicate that α-synuclein and hIAPP seeds strongly accelerate hIAPP fibril formation, Aβ seeds have a moderate effect, and H18R-hIAPP shows little to no influence. AFM and TEM revealed no major structural differences between hIAPP alone and seeded samples. Kinetic analyses with the webserver AmyloFit suggests distinct aggregation mechanisms depending on the seed type, with secondary nucleation or fragmentation predominating under different cross-seeding conditions. Together, these findings highlight the specificity of amyloid cross-seeding and suggest that structural compatibility between fibril surfaces and monomers determines the efficiency and mechanism of
heterologous aggregation.

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#10375

Study of ZnO Heterojunctions for Rapid Detection of Low-Concentration Gases: The Case of Ethanol and Ammonia

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Topics

Other

Communication type

Poster

Abstract

This study explores the performance of a gas sensor using zinc oxide (ZnO) as the sensitive material for the detection of vapors at very low concentrations. Thanks to its semiconducting properties, wide band gap, and high surface sensitivity, ZnO stands out as an ideal candidate for such applications. The material was synthesized using a method that yields nanostructures with a large specific surface area, promoting gas molecule adsorption and thus enhancing the sensor's sensitivity.

X-ray diffraction (XRD) characterization of ZnO confirmed the purity and crystallinity of the obtained phase. Detection tests were carried out under controlled conditions by exposing the sensor to low concentrations of ethanol and ammonia, down to 10 ppm. The results show excellent reactivity with very short response times: approximately 5 seconds for ethanol and 3 seconds for ammonia. In terms of sensitivity, the sensor demonstrated a response rate of 60% for ethanol and over 90% for ammonia, highlighting its high performance.

The marked selectivity towards ammonia compared to ethanol suggests a stronger chemical interaction between this basic gas and the active sites of ZnO. This interaction favors fast adsorption and desorption kinetics at the sensor's surface, contributing to both rapid and accurate detection.

These findings confirm the strong potential of ZnO in the development of sensitive, selective, and fast gas sensors, particularly valuable in safety, environmental monitoring, and medical applications, where the detection of trace gas concentrations is crucial.

Keywords: ZnO, gas sensor, low concentration, X-ray diffraction, fast response time, ethanol, ammonia, nanostructures

#10377

Impact du dioxyde de titane (TiO2) sur les propriétés structurales du polyéthylène basse densité (LDPE)

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Topics

Other

Communication type

Poster

Abstract

Les nanocomposites polymères, en particulier ceux à base de polyéthylène basse densité (LDPE) incorporant des nanoparticules inorganiques, suscitent un intérêt croissant en raison de l'amélioration significative de leurs propriétés mécaniques, optiques et thermiques. Parmi ces matériaux, le système LDPE/dioxyde de titane (TiO_2) se distingue par sa stabilité chimique, ses performances optiques et sa capacité à renforcer la matrice polymère.

Dans cette étude, l'incorporation de nanoparticules de TiO2 (0,5–5 % en poids) dans le LDPE a été réalisée par mélange en solution. Les analyses Raman ont montré une intensification des pics vibrationnels, notamment du mode d'étirement symétrique des groupes CH_2 ($\approx$1130 cm^{-1}), ainsi qu'un affinement des bandes indiquant une augmentation de la cristallinité. Ces résultats suggèrent une interaction efficace entre les nanoparticules et la matrice polymère.

Les analyses MEB ont révélé une dispersion globalement homogène des nanoparticules de TiO_2 ($\approx$100 nm, de morphologie pseudo-sphérique) au sein de la matrice de LDPE, malgré la présence de quelques agglomérats isolés. Ces nanoparticules jouent le rôle de centres de nucléation, ce qui favorise une meilleure organisation des chaînes polymériques et conduit à une augmentation de la cristallinité. Cette interaction se traduit par une amélioration des propriétés vibrationnelles et structurales du composite, tout en préservant son intégrité morphologique.

#10379

Langmuir lipid monolayers as models of E. coli membranes to study the internalization of lipid-oligonucleotide conjugates (LONs) in the context of antibiotic resistance

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Topics

Lipids and membrane proteins

Communication type

Oral

Abstract

Keywords: antibiotic resistance, *E. coli*, lipid Langmuir monolayers, Atomic Force Microscopy

With the rise of antibiotic resistance, the development of new therapeutic strategies is becoming an urgent global priority. Among the emerging alternatives, oligonucleotide-based approaches, particularly those involving targeted delivery, have gained increasing attention. In this context, a new class of molecules, termed Lipid-OligoNucleotide conjugates (LONs), has been designed[1]. These molecules combine a short interfering RNA (siRNA) sequence with two acyl chains similar to tails of membrane lipids, with the aim of silencing resistance genes such as those encoding β-lactamases enzymes responsible for the deactivation of third-generation cephalosporins in *Escherichia coli*[1]. The siRNA sequence is designed to block the enzyme synthesis by base-pairing to the complementary strand of mRNA.

To date, no study has explored the interaction of LONs with biological membranes, and the mechanism by which these molecules might penetrate bacterial cells remains entirely unknown. In this project, we aim to characterize the physicochemical interaction of LONs with simplified membrane models mimicking the outer membrane of *E. coli*, using Langmuir monolayers at the air-water interface. Preliminary results from surface pressure-area measurements and atomic force microscopy reveal that LONs interact more strongly with zwitterionic phospholipids than with anionic ones, leading to the formation of distinct domains within the monolayers. Further investigations will focus on the insertion, organization, and phase behavior induced by LONs at the molecular level.

Building on these model studies, LON-membrane interactions will also be investigated in a whole-cell approach using wild-type and genetically modified *E. coli* strains with altered membrane compositions, in order to bridge molecular-level findings with cellular-scale observations. These investigations will shed light on the internalization mechanisms of LONs, a key step toward overcoming intracellular resistance barriers. In the longer term, such insights may guide the development of adjunct therapies aimed at disabling bacterial defense systems and restoring antibiotic efficacy.

This project is funded by Agence Nationale de la Recherche (ANR-22-CE35-0015).

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#10401

Structural and biophysical characterization of the APE1-NPM1 complex

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Topics

Cancer

Communication type

Poster

Abstract

In order to maintain genomic integrity and prevent the emergence of mutations that can lead to various types of cancer, cells possess a wide range of mechanisms specialized in detecting and repairing DNA damage, each capable of targeting specific types of damage. These systems are particularly challenged – even overwhelmed – by commonly used chemotherapeutic agents, which bind to the DNA of cancer cells and induce irreparable damage, leading to apoptosis. Among the known mechanisms of chemoresistance, one involves an increase in the DNA repair capacity of tumor cells.

The protein nucleophosmin 1 (NPM1) has been identified as a key player in several DNA repair pathways, particularly base excision repair (BER). The BER pathway is essential for cells to restore the integrity of their genome in response to oxidative stress, such as that induced by metabolic activity, cancer treatments, or exposure to ionizing radiation. NPM1 regulates the stability, activity, and nucleolar localization of proteins involved in this pathway, including apurinic/apyrimidinic endonuclease 1 (APE1), an enzyme essential to the BER pathway.

However, despite the functional importance of the NPM1/APE1 complex, the molecular basis governing its formation remains poorly understood. Recent data indicate that (i) the expression of NPM1 and APE1 is greatly increased in various cancers, (ii) NPM1 and APE1 are associated with poor prognosis and constitute potential therapeutic targets, and (iii) NPM1/APE1 interaction contributes to the resistance of certain cancer cells to certain chemotherapy agents, particularly platinum salts.

This project aims to elucidate the regulatory function of NPM1 in the BER pathway and its role in cancer resistance, by investigating the molecular and structural basis of the NPM1/APE1 complex through an integrative approach combining activity assays, biophysical interaction measurements and structural studies.

#10435

Theoretical study of flavonoids involved in Alzheimer's disease

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Topics

Drug Design

Communication type

Poster

Abstract

Neurodegenerative diseases are mainly characterized by the progressive loss of neurons. Among them, Alzheimer's disease and Parkinson's disease are the most common. These pathologies are growing rapidly worldwide, with around 47 million people suffering from dementia in 2016[1,2]. Alzheimer's disease is characterized by an abnormal accumulation of amyloid-β plaques on the outside of neurons, as well as by the formation of neurofibrillary tangles inside nerve cells in different regions of the brain [3]. In the context of the study of the mechanisms governing the progression of Alzheimer's disease, we have observed that natural flavonoids act as effective inhibitors of acetylcholinesterase, reducing the secretion of ßA in neurons.

In this study, we aim to determine the interaction mode of the complex between acetylcholinesterase and flavonoids using molecular modeling methods. The complex with the lowest interaction energy will have better activity and therefore better inhibition.

Keywords: Alzheimer's disease, acetylcholinesterase, flavonoids ,ADMET, molecular modeling.

#10436

Structural studies of the AcrAB-TolC efflux pump from Escherichia coli in native vesicles

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

AcrAB-TolC is an efflux pump of Escherichia coli, a tripartite complex that contributes to antibiotic resistance by exporting a broad range of toxic compounds. So far, structural studies of this system have relied on artificial membrane models, which fail to reproduce the native lipid composition and the dual-membrane architecture of the bacterial envelope. To overcome this limitation, we are developing an approach to investigate efflux pumps directly within native vesicles derived from E. coli. As a proof of concept, we have reconstituted TolC in this native context and demonstrated that structural information can be obtained from purified vesicles. We are now working to enhance the resolution of TolC to better characterize its architecture and conformational dynamics. Building on this foundation, our ultimate goal is to resolve the complete AcrAB-TolC complex within native double-membrane vesicles, providing unprecedented insight into its organization and function in a physiological environment.

Structural characterization of an adhesion GPCR disordered c-terminal with AlphaFold

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Topics

Methods

Communication type

Poster

Abstract

The lack of experimental structural data has historically limited studies of intrinsically disordered regions (IDRs) and proteins (IDPs), despite their biological importance. Unlike folded proteins, IDRs/IDPs lack a single stable folded structure and rather populate heterogeneous conformational ensembles. Experimental techniques such as Nuclear Magnetic Resonance (NMR) and Small-Angle X-ray Scattering (SAXS) provide insights into their structure–function relationship, but the data are ensemble-averaged and difficult to translate into atomic-resolution structural ensembles. Molecular dynamics simulations are the standard tool for generating structural ensembles, but for IDPs they require sampling an impractically large conformational space. To address this, we recently developed a Bayesian framework, bAIes, which integrates AlphaFold2 predictions with a random coil model. This approach accelerates convergence and yields atomic-resolution ensembles consistent with both low- and high-resolution experimental data. Here, we applied bAIes to the C-terminal disordered tail of an adhesion GPCR, where mutations are linked to Usher2 syndrome, a rare disease causing combined hearing and vision loss. AlphaFold2 predicted stable helices in this region, a feature only partially supported by NMR data. In contrast, bAIes ensembles showed better agreement with experimental data than a pure random coil model, and performed best when soft, rather than hard, restraints were applied to the predicted helices. This indicates that helices occur only transiently, in contrast to AlphaFold2's static prediction. Despite their transient nature, these helices may be critical for partner binding through a folding-upon-binding mechanism.

#10462

New developments on SWING beamline

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Topics

Methods

Communication type

Poster

Abstract

Small-angle X-ray scattering of proteins in solution (BioSAXS) has emerged as a pivotal tool for biochemists and structural biologists, owing largely to the availability of high-throughput beamlines at synchrotron sources. Since its opening in 2008, the SWING beamline at synchrotron SOLEIL has been devoted in providing state-of-the-art for biological SAXS instrumentation [1]. This presentation highlights two notable enhancements on SEC-SAXS and direct injections setups.
The intense flux generated by synchrotrons can pose challenges for biological samples resulting in capillary fouling at the beam position. To address this issue, the SWING beamline introduces a Co-Flow system for SEC-SAXS, which sleeves the sample exiting the column within a buffer flow inside the capillary. This innovative solution prevents the sample fouling onto the quartz capillary under beam exposure.
Our previous direct injection system is incompatible with highly viscous samples. Indeed most of such the sample is lost within the tubing between the injection seat-needle and the SAXS capillary (wetting effect). To overcome this challenge, we undertook a completely new approach of our pipetting and injection system, incorporating a robotic arm with electronic pipettes. With this revamped system, samples are injected directly into the capillary, ensuring no sample loss. Furthermore, the new system enhances reproducibility of SAS measurement and significantly reduces the time of the complete measurement cycle (pipetting, measuring, washing, drying), down to less than 3 minutes for a 40 µL sample even for highly viscous ones.

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Annexes

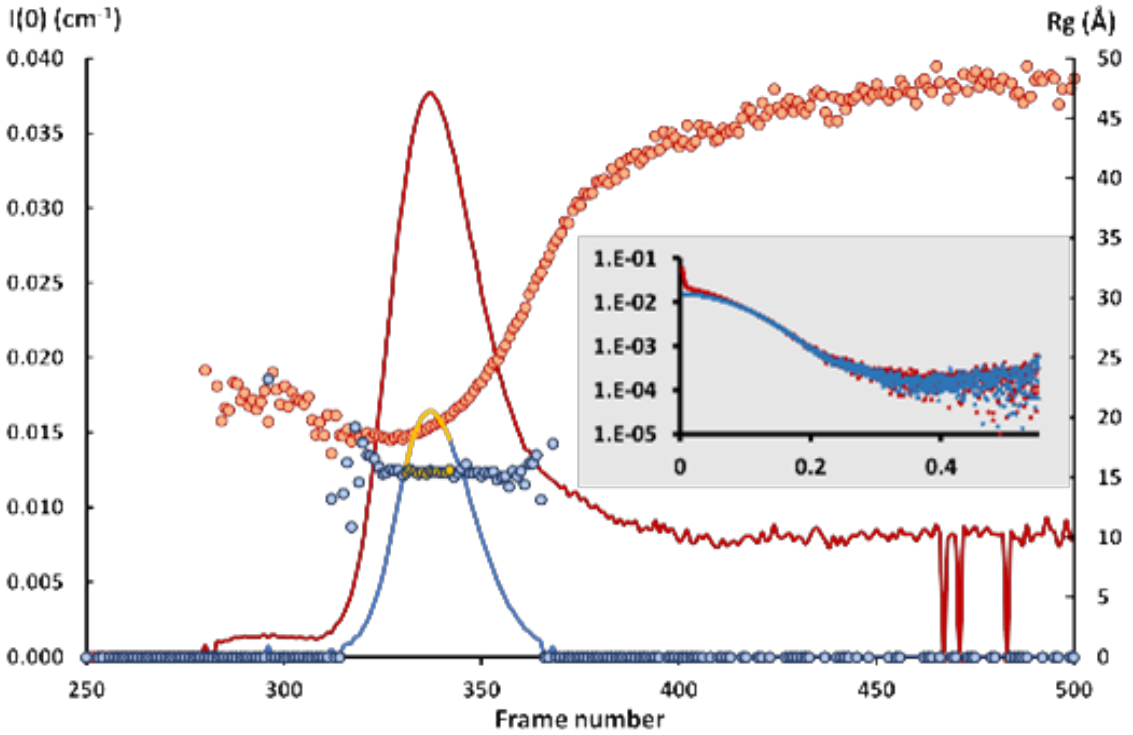

Figure 1: SEC-SAXS elution profiles of RNaseA sample in PBS buffer with (blue) and without (red) the CoFlow system. For each frame number, the Guinier analysis provides I(0) and Rg values shown as line and circles, respectively. In the inset, scaled averaged c

Figure 2: Robotic Arm installed on the SWING beamline.

#10507

Structure–Activity relationships of novel semi-synthetic pyridomycin derivatives

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Topics

Other

Communication type

Poster

Abstract

Structure–Activity relationships of novel semi-synthetic pyridomycin derivatives

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Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), is responsible for the deaths of 1.25 million people in 2023, including 161,000 HIV-positive individuals. Moreover, a quarter of the world's population is infected with the TB bacillus (WHO). MTB possesses a unique cell envelope rich in complex lipids, such as mycolic acids (MAs), which are very long-chain α-alkylated and β-hydroxylated fatty acids. These specific lipids constitute the most abundant component of the mycobacterial cell envelope and are essential for the virulence, impermeability and resistance of MTB.

The synthesis of MAs in mycobacteria involves two fatty acid synthases, FAS-I and FAS-II. FAS-I synthesizes (C16-18) acyl-CoA derivatives whereas FAS-II produces ultra-long meromycoloyl chains (C60-90). Unlike FAS-I, FAS-II involves mutliple enzymes, including the Enoyl-Acyl Carrier Protein Reductase, InhA. This enzyme catalyzes the NADH-dependent reduction of the double bond of 2-trans-enoyl, ensuring the elongation of the MAs carbon chain. InhA is the target of the most effective anti-TB drug, isoniazid (INH). However, INH acts as a prodrug that requires activation by the catalase-peroxidase KatG, producing an INH-NAD adduct that specifically inhibits InhA. Consequently, InhA still represents a relevant target for the development of novel direct antimycobacterial treatments.

Currently, treatments for patients infected with MTB are burdensome and lengthy. They involve a combination of four antibiotics (INH, rifampicin, ethambutol and pyrazinamide) for two months, followed by dual therapy with INH and rifampicin. Furthermore, the emergence of multi-resistant strains makes it very difficult to treat MTB infections. To address these limitations, pyridomycin, a natural product, emerged as a promising candidate. Pyridomycin directly inhibits MTB-InhA, overcoming established resistance and

making it an attractive candidate for drug development.

In this study, we present a structure activity relationships analysis of semi-synthetic pyridomycin analogs. The impact of pyridomycin analogs was then measured using in vitro and in vivo studies. High-resolution structural data demonstrate that pyridomycin and its analogs bind to the catalytic pocket of MTB-InhA, in the NADH binding site. As expected, co-crystal structures of InhA with pyridomycin derivatives showed the compounds bound in largely overlapping positions. Despite the structure similarity, several pyridomycin derivatives exhibit superior in vitro antibiotic activity and metabolic stability compare to the parent natural product.

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#10514

STRUCTURAL AND FUNCTIONAL STUDY OF THE PHOPR TWO-COMPONENT SYSTEM IN MYCOBACTERIUM TUBERCULOSIS

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Objectives: The PhoPR two-component system is essential in the virulence and intracellular growth of Mycobacterium tuberculosis (Mtb). Genomic studies reveal that natural mutations in this system emerged during evolution of mycobacterial species [1]. Some MTBC members have acquired compensatory mutations to preserve their pathogenic potential [2]. In collaboration with the "Molecular Mycobacterial Pathogenesis" team at IPBS, we are interested in the characterization of the PhoR histidine kinase to understand the signal transduction mechanisms of the PhoPR system and the adaptation of the pathogen to the intracellular environment [3, 4].

Methods: We investigate the full-length membrane PhoR protein using cryo-electron microscopy (cryo-EM) and X-ray crystallography. At the same time, using native mass spectrometry and PhosTag methods, we try to determine the parameters that activate PhoPR via phosphorylation.

Results: PhoR has been purified and stabilized using DDM and peptidiscs for structural analysis. Preliminary biophysical analyses were performed using mass photometry, dynamic and static light scattering, native mass spectrometry, and thermostability measurements (nanoDSF) to verify homogeneity and stability with detergents/peptidiscs. Domain crystallization was attempted but revealed unsuccessfull, and now cryo-EM and crystallization trials are ongoing on the full-length solubilized PhoR. Initial thermostability data suggest that PhoP is able of phosphorylation, but this remains to be confirmed for PhoR.

Conclusions: Refinement of purification conditions led to improved sample homogeneity, paving the way for cryogenic electron microscopy experiments towards solving the 3D structure of PhoR. At the same time, visualization of phosphorylation remains a real challenge.

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#10515

Tools for Mapping Virus-Host Associations at Metagenomic Scale

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Topics

Microbes

Communication type

Poster

Abstract

Viruses are ubiquitous parasites that exploit host cells across all domains of life for their replication. Viral infections have profound societal, ecological and environmental impacts, causing significant diseases in humans, animals and plants. While high-throughput sequencing (HTS) has revolutionised our understanding of microbial and viral diversity, our knowledge of which viruses infect which hosts remains limited, particularly when studying complex samples from patients or natural environments. We are developing a novel, scalable method to bridge this gap. This method will enable the identification of virus-host associations by creating and sequencing chimeric RNAs, formed by covalently linking host ribosomal RNAs (rRNAs) to the viral messenger RNAs (mRNAs) being actively translated within infected cells. Here we focus on the engineering of enzymes that permit this RNA proximity ligation step, employing protein engineering strategies. By identifying virus-host combinations in diverse environments, these new tools will generate new knowledge to advance both fundamental and translational research across ecosystems, extending findings from model organisms to the vast majority of virus-host systems that are currently inaccessible to laboratory study.

#10516

Dendrogenins: biosynthesis and interactions with the LXR? receptor

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Topics

Cancer

Communication type

Poster

Abstract

Breast cancer is the most common cancer among women in France and is also the most deadly [1]. Cholesterol, as a precursor of numerous lipids, plays a central role in many biological processes. Marc Poirot's team at the CRCT (Centre de Recherches en Cancérologie de Toulouse), highlighted the role of cholesterol in the progression of breast cancer tumors: it acts a precursor of 5,6-epoxycholesterols (5,6-EC) [2]. It has been shown that 5,6β-EC is enzymatically converted to oncosterone, a tumor promoter, while the 5,6α-EC stereoisomer, in the presence of nucleophiles, is converted into dendrogenins A, B or C (DDA, DDB, DDC) [3]. Among these, DDA results from the condensation of histamine with 5,6α-EC, catalysed by an unknown enzyme, named DDA synthase (DDAS). DDA is a natural metabolite, which acts as a tumor suppressor and is found only in healthy tissues and is absent in tumor cells [4].

We recently demonstrated through biological, biochemical, cellular, and crystallographic approaches that human glutathione S-transferase (hGSTA1) was the DDAS. The enzyme was characterized using biophysical techniques, and structural data were obtained by X-ray crystallography, including in the presence of 5,6α-EC analogues, of DDA and of DDC. The obtained structural data suggests that a distinct catalytic mechanism is at play in the biosynthesis of DDA or DDC. We aim at determining the molecular pathway that results in either DDA or DDC synthesis, as well as the capacity of isoforms of hGSTA1 (hGSTA2, hGSTA3) to perform similar reactions.

It has been shown that DDA's role in cell death by autophagy resulted from the interaction of DDA with the liver nuclear receptor beta (LXRβ) [4], leading to the expression of pro-autophagic factors. We aim at characterizing how DDA modulates the activity of LXRβ and its capacity to recruit co-regulators. We produced and purified the recombinant LXRβ in order to perform structural and biophysical studies of its interaction with dendrogenins, and to evaluate how this interaction affects coregulator binding.

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Microdialysis device to set up kinetic crystallography experiments

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Topics

Methods

Communication type

Poster

Abstract

The application of time-resolved crystallography to enzyme catalytic processes raises a number of is-sues in the preparation of experiments [1, 2, 3, 4]. In addition to crystallogenesis, it is necessary to verify the preservation of the enzyme activity in the crystalline form as well as adjusting a number of parameters con-trolling the reaction kinetics. Indeed, substrate diffusion times within the crystal matrix must be determined as well as the concentrations required to ensure that the different reaction states are captured. The possibil-ity to pre-calibrate most of these parameters at home-laboratory would increase the efficiency of the fore-seen kinetic experiments and the access to synchrotron (XFEL) instruments as well as dedicated platforms such as the icOS platform in Grenoble. For this reason, we have developed a 3D-printed support to allow mi-crodialysis with a reduced external volume.

Microdialysis also allows us to check the enzyme activity within the crystal compared to the soluble protein under the same conditions. This tool makes it possible to prepare kinetic crystallography experiments by determining the substrate concentrations and the diffusion time required for the experiment and by taking into account the relationship between the diffusion time and the density of crystallisation solutions. Microdi-alysis by reducing the required volume is cost effective in particular for precious protein samples and/or ex-pensive substrates. It also allows to multiply the measurement points.

The exploitation of this new tool will be illustrated through several examples such as substrate diffu-sion for two different enzymes but also pH changes for a fluorescent protein. Ultimately, we will also show that the tool can be exploited to study the activity of a membrane protein complex system that require the physical separation of the different key players in the reaction.

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The Magnaporthe oryzae MAX effector AVR-Pia binds a novel group of rice HMA domain-containing proteins

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Topics

Plants

Communication type

Poster

Abstract

Phytopathogenic fungi secrete effector proteins to promote virulence. MAX (Magnaporthe Avrs and ToxB-like) effectors form a sequence-diverse family sharing a conserved protein structure. AVR-Pia, a MAX effector from the rice blast fungus Magnaporthe oryzae, is recognised by the paired rice nucleotide-binding leucine-rich repeat immune receptors OsRGA4/OsRGA5 through direct binding to an integrated heavy metal-associated (HMA) domain in OsRGA5. Here, we identify previously unknown host targets of AVR-Pia: four HMA domain-containing rice proteins, belonging to the HMA Isoprenylated Plant Proteins (HIPPs) and HMA Plant Proteins (HPPs). AVR-Pia interacted with all four proteins, both in vitro and in planta, and bound their HMA domains with varying affinities. The crystal structure of AVR-Pia in complex with the HMA domain of OsHPP09 revealed the molecular details of the binding interface. Structure-guided mutagenesis of OsHPP09 identified a single point mutation which prevents AVR-Pia binding, providing a foundation for targeted engineering of HMA domains to evade effector binding.

#10523

Artificial protein scaffolds to facilitate the Cryo-EM structure determination of tricky proteins

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Topics

Other

Communication type

Poster

Abstract

The MIP team has designed a family of artificial proteins composed of alpha helices adapted from HEAT repeats (αReps). The highly-variable internal face of these αReps can be used to select specific binders for proteins of interest. This work is using these binders, fused with artificial protein trimers in order to attach the proteins of interest to a large protein scaffold.
Such complexes are more easily detected with Cryo-Electron Microscopy, which struggles to accurately detect smaller proteins (below 50 kDa).

As a proof of concept, this partnership with the I2BC CryoEM platform attempts to determine the structure of GFP and the tubulin dimer. Although these structures have already been solved, they are representative of challenges faced with Electron Microscopy and emerging technologies such as AlphaFold.
Once a generic workflow is established, these scaffolds could be adapted for any protein of interest provided that an appropriate αRep binder has been selected against it.

Annexes

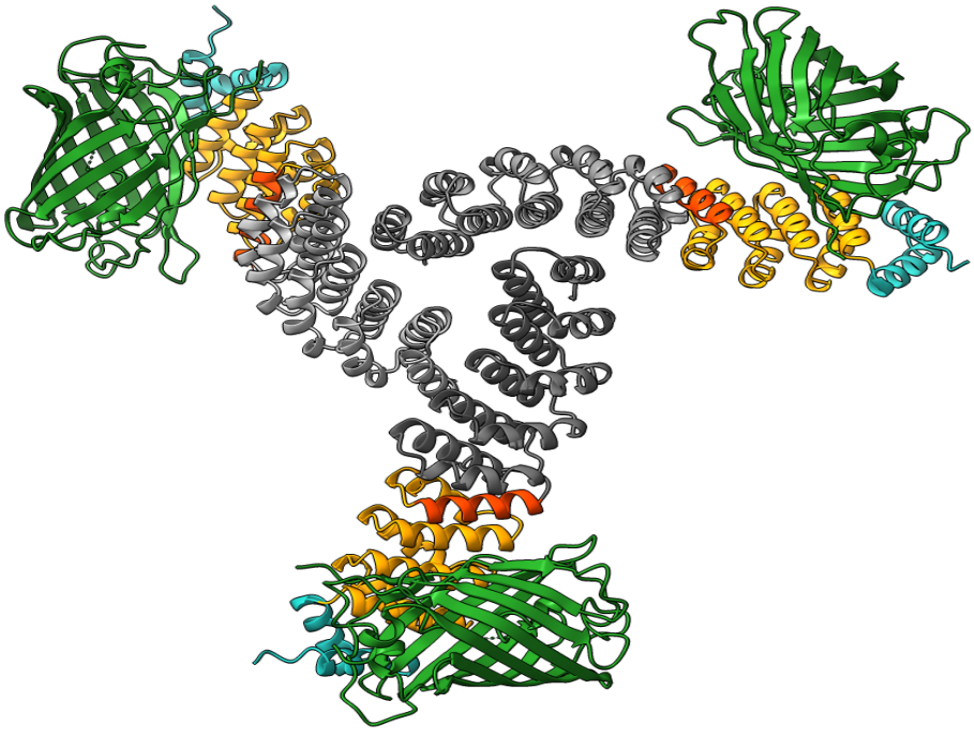

Figure 1

#10525

Structural Biology Plugins from the Structural Bioinformatics Library

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Topics

Methods

Communication type

Poster

Abstract

Over the past decade, we have developed reference algorithms
for various tasks in structural biology, namely
computing molecular surfaces and volumes,
modeling and comparing interfaces,
performing structural alignments and comparing structures,
decomposing structures into rigid domains,
or assessing the quality of AlphaFold predictions.

The corresponding tools are available in the
Structural Bioinformatics Library, see
https://sbl.inria.fr/applications/

To ease the utilization of these tools,
we recently developed a framework to
automatically generate plugins for VMD, Pymol, and web servers.

This poster (talk?), accompanied by a demo, will present these plugins.

#10526

Baeyer-Villiger MonoOxygenases from Mycobacterium tuberculosis : From Enzymatic Characterization to Structural Insights

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Topics

Microbes

Communication type

Poster

Abstract

Introduction : Tuberculosis (TB) remains a global health threat, with Mycobacterium tuberculosis (Mtb) being the leading cause of death from a single infectious agent and which is thought to affect one quarter of the world's population. The rise of antibiotic resistance to both first- and second-line anti-TB drugs underscores the urgent need for new TB treatments. Several currently available drugs to treat TB are prodrugs, thus enhancing bioavailability and stability, hence reducing toxicity and they require activation by specific Mtb enzymes. Ethionamide (ETH) and thiacetazone (TAC) prodrugs are activated by Baeyer-Villiger monooxygenases (BVMOs). However, in vitro research on these enzymes is hampered by protein purification challenges, while the precise enzymatic and structural characteristics of these BVMO remain poorly understood, with no structure solved experimentally to date.

Methods : This study aims at elucidating the enzymatic and structural properties of BVMO from Mtb to unravel the molecular mechanisms involved and allow the discovery of new prodrugs. We developed a purification protocol for two BVMO proteins using empiricial screening of conditions by biophysical techniques based on solubility, apparent molecular mass, polydispersity and thermostability of the target protein. We set a miniaturized in vitro enzymatic assay tailored for substrate and prodrug screening and generated models to correlate BVMO structure-function properties. We studied structural features of BVMO by various biophysical approaches including mass photometry, dynamic light scattering, X-ray crystallography and electron microscopy.

Results : The optimization of expression and purification conditions of BVMO led to improved purification yields with highly pure protein suitable for enzymatic and structural studies. Using a miniaturized enzymatic assay, we showed selectivity of purified BVMO for linear ketone substrates, confirmed by in silico docking of the substrates with models generated by homology. By adapting the miniaturized assay, we were able to determine the steady-state parameters of the BVMO with the prodrug ethionamide and its analog prothionamide. The purity of the samples allowed us to begin structural studies by crystallography. However purified BVMO do not behave as monomeric species and appear very polydisperse after purification, preventing from obtaining any crystal hit. The refinement of the purification conditions and the extensive use of biophysical tools improved the dispersity of the samples. Preliminary electron microscopy experiments showed homogenous distribution of oligomeric proteins, promising for further data acquisition to solve the 3D structure of a BVMO.

Conclusion : In optimizing expression and purification conditions for BVMO, we achieved high yields of pure protein suitable for enzymatic and structural analyses, revealing their selectivity for linear ketone substrates and enabling determination of steady-state parameters with prodrugs analogs. Despite initial challenges in crystallization due to polydispersity, refinement of purification conditions improved sample homogeneity, paving the way for structural characterization of BVMO using cryo-EM.

Tomas N and Leonelli D, Campoy M, et al., Bioinformatic Mining and Structure-Activity Profiling of Baeyer-Villiger Monooxygenases from Mycobacterium tuberculosis. mSphere. 2022

Spatial Organization and Dynamics of Mitochondrial Metabolic Complexes Revealed by Super- Resolution Microscopy and Biochemical Analysis

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Topics

Other

Communication type

Poster

Abstract

Mitochondria, as the central hub of cellular energy production, govern the spatial organization and dynamics of metabolic complexes that directly influence cellular function. Despite the importance of this organization, the nanoscale details and dynamic changes of these complexes in eukaryotic cells remain poorly understood. In this study, we employed super-resolution optical microscopy combined with specific protein labeling and biochemical analyses to investigate the organization and dynamic behavior of key mitochondrial metabolic pathway complexes. Our results revealed that electron transport chain complexes and ATP synthase are organized into distinct nanodomains, and this organization is dynamic in response to cellular metabolic changes. This combined approach enabled the identification of transient interactions and intermediate states of complexes, providing new insights into the relationship between cellular metabolism structure and function, which could serve as a foundation for studies on metabolic diseases and therapeutic strategies.

Keywords: Super-resolution microscopy, mitochondria, metabolic complexes, spatial organization, molecular dynamics, protein interactions, structural biology

#10530

Deciphering the role of the pseudokinase domain in the regulation of the GC-C Receptor

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

The receptor Guanylyl Cyclase C (GC-C) is expressed on the surface of intestinal epithelial cells, where it plays a central role in maintaining intestinal homeostasis and a healthy microbiome. GC-C activation is triggered by the binding of intestinal hormones (guanylin and uroguanylin) to the extracellular domain, which transduces signal across the membrane to the intracellular pseudokinase and guanylyl cyclase domains. Activated GC-C produces the second messenger cGMP, which activates downstream ion channels responsible for ion and water secretion, thereby facilitating intestinal transit1.

GC-C is the target of a toxin from enterotoxigenic E.coli, a WHO top-priority pathogen causing acute traveler's diarrhea2. GC-C dysregulation is implicated in severe human diseases such as meconium ileus, chronic constipation, inflammatory bowel diseases (Crohn's diseases) and colorectal cancer (the second leading cause of cancer-related deaths worldwide)1. Despite its physiological and clinical importance, the molecular mechanisms underlying GC-C activation remain poorly understood. Here, we investigated how the pseudokinase regulates GC-C activity and how ATP binding2 potentiates ligand-induced activation.

To this end, we determined the cryo-EM structure of the human GC-C intracellular domain bound to ATPγS at 3.3 Å resolution. The structure reveals a unique mode of ATP coordination (distinct from canonical kinases) and an unprecedented conformation of the pseudokinase domain that controls the rotation of the C-terminal guanylyl cyclase domain.

These findings provide the first structural insights to GC-C intracellular architecture and uncover the molecular basis of its allosteric regulation of cGMP synthesis. Our work substantially advances understanding of GC-C function and paves the way for new therapeutic strategies targeting GC-C-related diseases affecting millions worldwide.

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#10531

The yxiD gene from Bacillus subtilis 6633 encodes a polymorphic toxin with tRNase activity and is neutralized by the cognate immunity protein YxxD

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Topics

Microbes

Communication type

Poster

Abstract

Polymorphic toxin systems (PTS) are widespread and play an important role in bacterial competition and shaping communities. However, these systems are less studied in Gram-positive bacteria. Here, we structurally and functionally characterized YxiD-YxxD, a predicted member of PTS from Bacillus subtilis 6633. Using growth curve assays, we established that YxiD-YxxD codes for a toxin-immunity protein pair. We determined 1.7 Å resolution crystal structure of the YxiDCTDH528A (C-terminal domain of YxiD) toxin bound to its cognate immunity-protein YxxD. Structure revealed that the toxin adopts a Barnase/EndoU/colicin/RelE (BECR) fold, a characteristic of RNase toxins and immunity protein sterically occludes binding of molecular substrate to neutralize toxin. Structural and other biophysical studies revealed that YxiDCTD-YxxD forms a stable 1:1 stoichiometric complex with KD of ~9.4 nM. RNA Sequencing experiments revealed that expression of toxin results in downregulation of several tRNAs and essential genes involved in cell wall biosynthesis resulting in cellular toxicity. We further demonstrate that YxiDCTD is a metal ion dependent tRNase that cleaves several tRNAs. Taken together, our study provides the structural basis for YxiD neutralization by cognate immunity protein YxxD and establishes YxiDCTD toxin as a metal-dependent tRNase.

#10532

Structural Basis of Calcium-Dependent Ligand Recognition by Dendritic Cell Immunoreceptor (DCIR) in murine and Human orthologs: High-Resolution Crystallographic Insights into N-Glycan Specificity.

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Topics

Microbes

Communication type

Poster

Abstract

Structural Basis of Calcium-Dependent Ligand Recognition by Dendritic Cell Immunoreceptor (DCIR) in murine and Human orthologs: High-Resolution Crystallographic Insights into N-Glycan Specificity.

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Dendritic Cell Immunoreceptor (DCIR), also known as CLEC4A, is a type II transmembrane C-type lectin receptor that has been increasingly recognized for its pivotal role in modulating immune responses during both infectious and non-infectious inflammatory diseases. While extensive research has highlighted its significance in processes such as antigen presentation, immune cell activation, and the regulation of inflammatory cascades, the precise structural determinants that underpin its calcium-dependent ligand recognition have, until now, remained elusive and poorly characterized at the molecular level.

In the present study, we report the successful determination of the high-resolution crystallographic structures of the extracellular carbohydrate recognition domains (ECDs) of both murine and human DCIR. Our analyses reveal that both orthologs adopt the canonical C-type lectin fold, a highly conserved structural motif comprising a characteristic double-loop region, two α-helices, and a β-sheet scaffold, all of which are further stabilized by a network of conserved disulfide bridges. Intriguingly, within each structure, we have identified a single, well-defined calcium-binding site, which is coordinated by a set of evolutionarily conserved residues located within the EPS-like motif a feature known to be critical for the carbohydrate-binding properties of C-type lectins.

Moreover, through the co-crystallization of human DCIR with a biantennary N-glycan, we have been able to capture, at atomic resolution, the specific and intricate interactions that occur between the complex biantennary N-glycan and the carbohydrate recognition domain of the receptor. These interactions not only shed light on the molecular mechanisms that govern carbohydrate recognition by DCIR but also provide a structural rationale for the observed ligand specificity.

Collectively, these findings significantly advance our understanding of the calcium-mediated recognition processes employed by DCIR and offer unprecedented molecular insights into the specificity of its interactions with N-glycan ligands. Such structural information is expected to pave the way for the rational design of novel therapeutic interventions targeting DCIR-mediated immune responses.

#10534

Molecular determinantS driving Bcl-xL membrane insertion – exploration of its structural plasticity in solution and in nanodiscs

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Topics

Cancer

Communication type

Poster

Abstract

Multi-domain Bcl-2 family proteins share the ability to form dimers and oligomers, regardless of their pro- or anti-apoptotic activity. A remarkable trait is that homotypic interactions (pro-pro and anti-anti) and heterotypic interactions (pro-anti) are largely documented, and elucidating generalizable and case-specific structural rearrangements is still a timely quest. How such higher organization plays in their respective survival / death functions and in their interaction with membranes has repeatedly been investigated.

Looking into anti-apoptotic Bcl-xL, essentially engineered/truncated proteoforms lacking the disordered loop and/or the hydrophobic C-terminal helix have been used as proxy of the full length protein to elaborate on structural transitions and intermediate states between monomers and oligomers prior membrane insertion. Here, we report on full length (FL) human Bcl-xL (aa 1-233), either purified from bacteria or produced in a cell-free system. We discovered that both the disordered loop and the C-terminal helix are potent contributors to Bcl-xL structural plasticity.

We first considered the protein in solution, and found that conditions conducive to Asn deamidation in the disordered loop drive FL Bcl-xL to arrange in redox-dependent dimers that bridge the unique Cys151 from two monomers. However, this arrangement differs from the 3DDS previously reported with C-terminal truncated proteoforms because unlike the latter, FL redox dimers also form spontaneously: this indicates that the C-terminal helix lowers the energetic barrier for dimerization compared to truncated/engineered proteoforms.

We further explored the protein in the presence of synthetic membranes. The use of nanodiscs revealed unexpectedly that Asn deamidation in the unstructured loop impairs membrane insertion. This effect is not observed when Asp deamidomimic mutants are used, pointing instead to IsoAsp as molecular determinants preventing membrane insertion. Finally, we found that dimerization is not a prerequisite for membrane insertion. Instead, FL Bcl-xL forms adducts in nanodiscs that are not redox-dependent. Combined to cellular experiments showing that C151 mutation does not modify Bcl-xL survival functions, this suggests that the functional unit in membranes is monomeric Bcl-xL.

Altogether, our work identifies the disordered loop and the C-terminal hydrophobic helix as major regulators of Bcl-xL transitions states in solution and in membranes. Our data support that the natural plasticity exhibited by purified Bcl-xL is chaperoned in cells to prevent the structural transition leading to cystein-dependent dimers, and show that deamidation is a molecular damage that impairs membrane insertion.

Biophysical approaches to unravel binding mechanisms of hnRNP-A1 protein and G-quadruplex DNA in the kras promoter

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Topics

RNA/DNA

Communication type

Poster

Abstract

Mutations in the kras oncogene are direct causes for aggressive cancer development, notably lung and pancreatic adenocarcinoma. Studies suggest that the extremely G-rich DNA sequence within kras promoter form secondary structures known as G-quadruplexes (G4s). Those could act as platforms for the recruitment of ribonuclear proteins, participating in the regulation of kras. Among others, the protein hnRNP-A1 and its derivative UP1 have been shown to target and unwind the G4 structure formed just upstream of kras transcription starting site (the so-called “near G4”). This G4 has been thoroughly characterized in our lab and was proved to adopt two distinct conformations with parallel topologies. However, the kras promoter contains two other G-rich motifs further away of the TSS (the “mid” and “far” G4s), that have shown to be pivotal in the promoter regulation. The aim of my study is twofold:

1. Perform biophysical and structural characterization of the “mid” and “far” G4s
2. Monitor the interaction of the “near”, “mid” and “far” with UP1 to understand how the protein binds and unfolds G4s

For that, we use a large panel of biophysical methods including CD, BLI, ITC, UV-melting to obtain insights on structure of G4s and interactions with UP1, and NMR that provides structural data at the atomic level. Using 2D NMR spectroscopy, we obtained results that suggest that “mid” and “far” G4 are more polymorphic then “near”, and most probably they adopt different conformations in dynamic equilibrium. In addition, we demonstrated that UP1 is able to bind to the three G4s of kras promoter with distinctive features, including different sensitivities to UP1 inhibitors. Altogether, those results will allow to decipher the complexity of the molecular mechanisms in kras expression, opening new horizons for targeted therapies.

Investigating membrane nanodomain association of RocS in Streptococcus pneumoniae

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Chromosome segregation in the human pathogen Streptococcus pneumoniae relies on the membrane-binding protein RocS, which anchors chromosomal DNA to the cell membrane. Although RocS is small, it functionally compensates for the absence of both the Min and nucleoid occlusion system. However, the molecular basis of its membrane targeting has remained unclear. Because RocS activity is tightly regulated in space and time, we hypothesized that a conserved linker region modulates its interactions with lipids. Supporting this idea, mutational analysis showed that altering a single glycine residue significantly disrupts chromosome segregation and affects membrane properties.

To understand how RocS interacts with the membrane, we employed MAS and wide-line NMR together with real-time AFM imaging. NMR analysis revealed that the membrane anchor folds into a short membrane-inserted kink-helix motif connected to an extended linker, with membrane insertion locally perturbing lipid packing. The fluidity of the lipid bilayer, which is modulated by temperature, in turn influences the interactions of the anchor. At the mesoscale, AFM imaging showed that the anchor selectively associates with lipid nanodomains, clustering into discrete foci. Together, these findings provide a mechanistic explanation for RocS nanodomain targeting and illustrate how a highly conserved membrane-targeting motif mediates specific lipid interactions. Beyond S. pneumoniae, this work suggests general principles for how small membrane-binding domains coordinate DNA-membrane interactions in other conserved proteins.

Repair of the 8-oxoguanine lesion in a DNA:RNA hybrid context by DNA N-glycosylases

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Topics

RNA/DNA

Communication type

Poster

Abstract

Over the oxidatively DNA base lesions (1), 8-oxoguanine (GO) is the most prevalent and mutagenic lesion associated with G:C to T:A transversions. Thus, if not repaired, GO can contribute to carcinogenesis, aging, and degenerative diseases (2,3). To counteract these harmful effects, cells have evolved a highly conserved and efficient repair mechanism call the base excision repair (BER) pathway, initiated by specific GO-DNA N-glycosylases (4,5,6). The catalytic activity of these enzymes involves the cleavage of the N-glycosidic bond between GO and the deoxyribose sugar, resulting in the formation of an apurinic/apyrimidinic (AP) site. AP site is subsequently processed by AP endonucleases/AP lyases, DNA polymerases, and DNA ligases to complete the repair process. The GO-DNA glycosylases remove GO in double stranded DNA but in vivo emerging evidences indicate that GO is also found localized at G-quadruplex or DNA:RNA hybrid structures and participate in broader biological processes including transcriptional regulation, apoptosis and immune system modulation (7,8,9). In this study, we explore the ability of eukaryotic and prokaryotic GO-DNA glycosylases from the HhH (OGG1, OGG2 and AGOG) and H2TH (Fpg) structural superfamilies to recognize and remove GO in DNA:RNA hybrids (GO being in DNA strand).

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#10538

Bcl-xL insertion into membranes is assisted by additive interactions involving two domains of Bak – In vitro advance enabled by cell-free synthesis and nanodiscs

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Topics

Cancer

Communication type

Poster

Abstract

Programmed cell death (apoptosis) is tightly regulated by interactions within the Bcl-2 family of proteins, which includes both pro-survival members like Bcl-xL and pro-apoptotic effectors such as Bak. Bak promotes mitochondrial outer membrane permeabilization, while Bcl-xL counteracts this effect via direct interaction through canonical BH3-binding motifs. In this study, we employed a cell-free protein synthesis system combined with nanodiscs to express full-length Bak and Bcl-xL, without tags or detergents. This allowed us to investigate their membrane insertion properties and interactions in a membrane environment.

We demonstrate that while Bak inserts efficiently (100%) into membranes when expressed alone , Bcl-xL shows only partial insertion (~60%), which is significantly enhanced upon co-expression with Bak. This cooperative insertion involves both the canonical BH3-groove interaction and an interface mediated by the C-terminal hydrophobic helices of both proteins. Furthermore, mutations in the BH3-binding groove of Bcl-xL, commonly used in functional cellular studies, were found to impair membrane insertion, highlighting a potential misleading factor in the interpretation of their effects.

Altogether, our findings reveal a novel cooperative mechanism between Bak and Bcl-xL, shedding new light on the dynamic regulation of their membrane insertion. These insights are crucial for the design of therapeutics targeting Bcl-2 family proteins in cancer.

#10539

Biophysical approaches to unravel binding mechanisms of hnRNP-A1 protein and G-quadruplex DNA in the kras promoter.

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Topics

RNA/DNA

Communication type

Poster

Abstract

KRAS is one of the most frequently mutated oncogenes in human cancers and is considered largely “undruggable,” making it a critical challenge for therapeutic intervention. Its transcription is regulated by guanine-rich DNA sequences in the promoter region that can fold into unusual secondary structures called G-quadruplexes (G4s), which act as molecular switches. These G4s are recognized by several nuclear proteins, including Myc-associated zinc finger protein (MAZ, a transcription factor), Upstream-binding protein 1 (UP1, a fragment of hnRNP A1, a DNA-binding regulator), Poly(ADP-ribose) polymerase 1 (PARP-1, a DNA repair enzyme), and apurinic/apyrimidinic endonuclease 1 (APE1). These proteins are known to interact with G4s, but how they coordinate to regulate KRAS transcription and how G4s function as molecular platforms remains largely unknown. This project aims to uncover the mechanisms through which G4 DNA structures and their associated proteins regulate KRAS transcription and how this regulation is modulated by DNA modifications or chemotherapeutic agents. Using different DNA G4 constructs and recombinant proteins, we use integrative structural biology approaches, including 2D NMR, cryo-electron microscopy and complementary biophysical techniques such as single-molecule FRET and isothermal titration calorimetry to resolve the architecture, dynamics, and interactions of G4–protein complexes. In addition, we probed how certain protein and DNA ligands influence these interactions, and reshape the global fold of the protein-DNA complexes. The project addresses a major gap in cancer biology and may provide innovative strategies for therapeutic intervention, not only for KRAS but also for other proto-oncogenes regulated by promoter G4s.

Intrinsically Disordered Protein ensemble refinement in the era of Alphafold

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Topics

Methods

Communication type

Poster

Abstract

The rise of biomolecular structure prediction AI tools such as Alphafold has revolutionized the field of structural biology. Despite an extensive characterization of these AI tools, their ability to provide accurate information on disordered proteins remains unclear. Here, we use bAIes, a new Bayesian framework that combines AI information and molecular dynamics force fields to explore the capabilities of Alphafold2 to accurately describe structural ensembles of Intrinsically Disordered Proteins (IDPs). We derive structural ensembles of multiple different IDPs featuring distinct structural properties using bAIes on a random coil model and we methodically assess the agreement of calculated NMR and SAXS observables obtained from the simulations with experimental data. We show that the agreement with experiments is highly dependent on the accuracy of the AI model. We find that the agreement of the generated ensembles with respect to experimental data is comparable to that of that of state-of-the-art force fields despite requiring only a fraction of the computational cost. We also highlight the limitations of Alphafold-2 for ensemble generation and the importance of various types of experimental data to validate structural ensembles. Disorder is important in many biological processes and predicting atomistic-detailed ensemble properties is therefore valuable. This study paves the way to a better understanding on how to integrate AI methods and experimental data for an accurate structural description of IDPs.

#10542

A ubiquitin-like protein controls assembly of a bacterial Type VIIb secretion system

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Topics

Microbes

Communication type

Poster

Abstract

Type VII secretion systems (T7SS) are crucial bacterial nanomachines that mediate interbacterial competition and hostpathogen interactions in Gram-positive bacteria. Despite their importance, the structural basis for assembly and substrate transport in T7SSb, a widely distributed T7SS variant, remains poorly understood. Here, we present the cryo-EM structure of the T7SSb core complex from Bacillus subtilis, revealing how a ubiquitin-like protein, YukD, coordinates assembly of the secretion machinery. YukD forms extensive interactions with the central channel component YukB and promotes its association with the pseudokinase YukC, creating a stable building block for channel assembly. Using microscopy and competition assays, we demonstrate that YukD is essential for proper T7SSb complex formation and contact-dependent bacterial killing. Structural modeling suggests this YukD-dependent assembly mechanism is conserved across diverse Gram-positive bacteria. Our findings reveal how bacteria have adapted a ubiquitin-like protein as a structural regulator for assembling a large secretion complex.

Keywords: Type VII secretion systems (T7SS), Cryo-EM, interbacterial competition

#10543

Structure and fonction of the T7SSb in Bacillus subtilis

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Topics

Microbes

Communication type

Poster

Abstract

The Type VIIb secretion system (T7SSb) of Bacillus subtilis represents a recently identified multiprotein machinery thought to mediate interbacterial interactions and secretion of effector proteins. Despite its biological significance, the structural organization and molecular mechanism of this system remain largely unexplored. In this work, we employ a combination of biochemical purification, cryo-electron microscopy, and complementary biophysical approaches to investigate the architecture and assembly of the T7SSb complex. This study aims to provide a structural framework for understanding how Gram-positive bacteria deploy T7SSb to coordinate competitive behaviors within microbial communities.

#10544

The nucleating agents, crystallophores, to boost your crystal production.

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Topics

Methods

Communication type

Poster

Abstract

The crystallophore is a lanthanide complex combining phasing and nucleating properties [1], successfully exploited to produce crystalline forms free of crystal defects often encountered by crystallographers such as low-resolution diffracting samples or crystals with twinning [2], to generate crystals can from enriched fractions containing several proteins [2] leading to the structure determination of a protein complex [3] and to induce nucleation directly from the protein solution, as exemplified by the crystallization of hen egg white lysozyme in water [4].

Time-resolved Serial Crystallography (TR-SX) allows to obtain structural dynamics information and observe biological macromolecules in action by capturing transient intermediates along a biological pathway. [5–8] From an experimental point of view, serial crystallography brings new constraints on crystal preparation as it intrinsically requires a large amount of samples to make sure to collect a complete diffraction data set. Moreover optimal time-resolved experiments require crystalline samples with a narrow size distribution in order to ensure a uniform triggering of the reaction under study through the entire crystal volume.

The nucleating properties of the crystallophore have been challenged for the production of crystals with the appropriate size for either SX experiments or electron diffraction of 3D nanocrystals [9]. Moreover, these crystals were generated in the minute time-scale opening new opportunities in TR-SX. We will also show the input of crystallophore variants bearing chemical modifications to expand the possibility to control the number and size of crystals [10]. Finally, to facilitate crystal detection, a crystallophore with optimized imaging property completes the toolbox [11].

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#10545

Structural observations of Vibrio cholerae XerCD complex on DNA

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Topics

Microbes

Communication type

Poster

Abstract

Chromosome dimers form in around 10 % of the cells per generation in bacteria. If left unresolved, they remain trapped between the two daughter cells during division, which leads to chromosome shearing and eventually cell death. Chromosome dimers are separated by the addition of a crossover at a specific site, dif, by two highly conserved tyrosine recombinases, XerC and XerD. Numerous bacterial viruses exploit their host's Xer machinery to integrate in their host's genome. This includes viruses that provided the cholera toxin genes to pandemic strains of Vibrio cholerae.

XerC and XerD form heterotetrameric nucleoprotein complexes, composed of two target sites and a pair of each of the two recombinases. XerC and XerD act successively to exchange a specific pair of strands. Two recombination pathways can be defined depending on whether XerC or XerD initiates the process. Chromosome dimer resolution is initiated by XerD. This requires a direct contact with the extreme C-terminal domain of a cell division protein, FtsK. Bacteriophages can bypass the FtsK requirement by utilising the basal FtsK-independent activity of XerC and/or by producing their own Xer activation factor.

Thirty years of genetic and molecular research has yielded a deep understanding of Xer recombination. However, structural understanding of the process is still lacking. This is mainly due to the poor solubility and tendency to aggregate of XerC and XerD. Our recent work enabled homogeneous purification of Vibrio cholerae XerCD-dif nucleoprotein complexes at a scale suitable for structural characterization by X-ray crystallography and Cryo-electron microscopy studies. Buiding on previous genetic and molecular biology knowledge on the Xer recombinases, we assembled XerCD-dif nucleoprotein complexes trapped at various stages of the recombination reaction. We obtained CryoEM models of the synaptic complex using wild-type XerC and XerD proteins, the XerD-cleaved complex using XerC and a hyperactive XerD-FtsK fusion on a nicked dif site and the XerC-cleaved complex at resolutions between 2.3 Å and 2.7 Å. We also obtained the first model of wild-type XerCD-dif Holliday-junction intermediate at 3 Å by Xray-crystallography.

A Novel Approach to Study Paired NLRs

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Topics

Plants

Communication type

Poster

Abstract

Nucleotide-binding and leucine-rich repeat (NLR) proteins are crucial intracellular immune receptors that detect pathogen infection by recognizing effectors and trigger a hypersensitive response to counteract infection. NLRs share a common structural organization comprising a signaling N-terminal domain, a central nucleotide-binding domain and a C-terminal leucine-rich repeat domain. NLRs can function as singletons, in exclusive pairs consisting of a sensor and an executor, or in networks, depending on how detection and immune response activation are partitioned among different NLRs. The recent progress in protein production combined with cryo-Electron Microscopy and AlphaFold modelling enabled us to understand the molecular detail of NLR activated state, the active complex capable of triggering the immune response. Unfortunately, we are still lacking important knowledge about the inactive protein and so, their activation mechanism.

Among this diverse group, the NLR pair RGA4 and RGA5 from Oryza sativa have been extensively studied by our collaborators from the PHIM Institute. This NLR pair detects the blast effectors AVR-Pia and AVR1-CO39. The executor NLR RGA4 is auto-active, but is kept in an inhibited state by the sensor NLR RGA5 until the latter recognizes a cognate effector via an integrated heavy metal-associated (HMA) domain. Given the challenges in expressing and purifying NLR proteins, we developed an in cellulo assay based on Fluorescence Cross-Correlation Spectroscopy (FCCS) to characterize RGA4-RGA5 interactions. Using AlphaFold modeling and this novel biochemical approach, we aim to determine the different complexes formed by these proteins, their stoichiometry and interactions surfaces involved in maintaining RGA4 in an inactive state in the absence of the cognate effector.

#10577

ClpV ATPase: Structure and Role in Type VI Secretion System recycling

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Type VI secretion systems (T6SS) are contractile bacterial nanomachines that mediate interbacterial competition and virulence in Gram-negative bacteria. Following contraction and toxin delivery, the T6SS sheath must be disassembled by the AAA+ ATPase ClpV to enable component recycling and system reactivation. However, the late stages of T6SS function, particularly sheath disassembly and recycling mechanisms, remain poorly characterized. ClpV is recruited specifically to contracted sheaths, where TssC N-terminal helices become accessible for interaction, but the structural basis for this process is largely unknown. Here, we present the first cryo-EM structural analysis of ClpV from enteropathogenic E. coli. The ClpV structure reveals the ATPase adopts a hexameric open-ring conformation with flexible N- terminal domains that enable binding to TssC subunits of the contracted sheath. This architecture provides initial insights into how ClpV recognizes its substrate and positions itself for disassembly. These findings represent a first step toward understanding the molecular mechanisms underlying T6SS recycling and regulation of this important bacterial weapon system.

#10628

Plant-Derived Monoterpene Mixture Disrupts Feeding and Reproduction in Drosophila melanogaster

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Topics

Plants

Communication type

Poster

Abstract

Plants produce a diverse array of bioactive compounds that contribute to their defense against herbivores and pathogens. Among these, essential oils—rich in monoterpenes—have attracted attention for their eco-friendly insecticidal properties. Monoterpenes, naturally occurring in aromatic plants, can influence insect behavior and physiology, offering potential alternatives to conventional chemical pesticides.

This study investigated the effects of a 24-hour fumigant exposure to a binary mixture of two monoterpenes on adult Drosophila melanogaster, focusing on feeding and reproductive behaviors. Adult flies were exposed to controlled concentrations of the monoterpene mixture via fumigation for 24 hours. Feeding behavior was assessed using capillary feeding assays, while reproductive effects were evaluated by measuring oviposition rates and offspring viability compared to untreated controls.

Fumigant exposure significantly reduced feeding activity, suggesting an appetite-suppressant or irritant effect of the monoterpene mixture. Exposed flies also exhibited a marked decrease in egg laying and lower progeny viability, indicating adverse effects on reproductive capacity. These results demonstrate that exposure to monoterpene combinations can disrupt key behaviors essential for survival and population maintenance.

Our findings highlight the potential of plant-derived monoterpene mixtures as eco-friendly fumigants capable of simultaneously affecting insect feeding and reproduction. Understanding these behavioral disruptions may guide the development of botanical pest control strategies targeting multiple life-history traits, thereby contributing to sustainable and environmentally conscious pest management.

#10819

Structure of the type IV pilus from Streptococcus sanguinis

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Topics

Lipids and membrane proteins

Communication type

Poster

Abstract

Type 4 filaments (T4F) are ubiquitous prokaryotic nanomachines driving critical functions like adhesion, motility, and DNA uptake. While structural data abounds for Gram-negative bacteria (diderms), the architecture of T4F in Gram-positive species (monoderms) has remained elusive. Here, we present the 3.7 Å resolution cryo- electron microscopy structure of the Type 4 Pilus (T4P) from the Gram-positive model Streptococcus sanguinis. This structure reveals the first heteropolymeric pilus architecture, composed of two major pilins (PilE1 and PilE2) assembled stochastically. Our atomic model highlights both universal and unique features. We confirm that the "melted" (unfolded) portion of the N-terminal α-helix is a universal trait of bacterial T4F, conserved even in phylogenetically distant monoderms. Uniquely, the filament cohesion in S. sanguinis relies on a "Velcro-like" mechanism, where the C-terminal tail of one subunit locks into the αβ -loop of the next via electrostatic interactions. Finally, we provide a complete integrated model of the pilus capped by a tip complex of minor pilins (PilA, PilB, PilC). This work establishes S. sanguinis as a key model for understanding T4F biology.

Keywords: Streptococcus sanguinis, Type IV pilus, Cryo-electron microscopy

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