FRISBI – 8th Users Meeting 2024

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FRISBI – 8th Users Meeting 2024 : Scientific Speakers

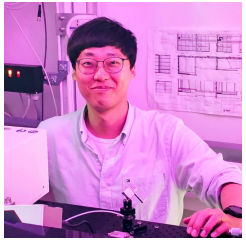
Changqing Li *(IGBMC, Illkirch)*
A novel YDDFXF linear motif mediates docking of IKK catalytic dimers to NF-kB substrates: structural and functional insights

Lionel Ballut *(IBCP, Lyon)*
Filamentation control of the activity of a nucleotidase (Isn1) from Nakaseomyces glabrata involved in NAD synthesis

Stéphane ROCHE *(i2BC, Paris-Saclay)*
CryoEM structure and assembly mechanism of the DNA gatekeeper complex of bacteriophage SPP1

Dominique HOUSSET *(IBS, Grenoble)*
3D electron diffraction: new opportunities in structural biology, material science and chemistry

Sihuyn SUNG *(EMBL, Grenoble)*
Automated crystallographic pipelines for new structures and drug design



FRISBI – **Working Group**

WG - **Biophysical & Spectroscopy**

Coordinators : C. Mas and J. Ménétrey

WG - **Sample Preparation**

WG - **Nuclear Magnetic Resonance**

WG - **Electron Microscopy**

WG - **Crystallization & X-ray Crystallography**

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WG – **Biophysical & Spectroscopy** (coordinators C. Mas and J. Ménétrey)

Dark blue: Leader platforms
Light blue: Technology available

Updated Nov24

	Grenoble	Marseille	Paris-Saclay	Strasbourg	Montpellier
Structure & Oligomers					
CD					
SEC-MALLS					
Anal Ultracent					
Automated PEAQ DSC					
FSEC					
NanoDSF					
Mass Photometry					
Protein Interactions & mobility					
ITC					
SPR					
Bio-Layer Inteferometry					
Microscale Thermophoresis					
FIDA	2025				
SwitchSense /Helix					
Spectroscopies					
EPR					
EPR high field					
FTIR					
RAMAN					
Super resolution microscopy					
Electronic Spectroscopy					
in crystallo optical spectroscopy	icOS Lab				
SAXS in house					
SAXS in house					Data
Binders					
Nanobodies					
Alpha-Rep					

IBS, Grenoble
Caroline MAS
Antoine ROYANT

CBS, Montpellier
Frederic Allemand

IGBMC, Strasbourg
Catherine BIRCK
Natacha ROCHEL

AFMB, Marseille
Anaïs GAUBERT
Maria MATÉ-PÉREZ

I2BC, Paris-Saclay
Stéphanie MARSIN
Agathe URVOAS
AnnaMaria QUARANTA

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Spectroscopy facility

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Binders

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Binders

Spectroscopy facility

WG – Biophysical & Spectroscopy : What happened in 2024

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Equipment acquisition

New EQUIPMENTS

- Laser Raman Femto-second (Paris-Saclay)
- Mass photometry (Strasbourg)

Updated EQUIPMENTS

- DLS (Montpellier)

WG – Biophysical & Spectroscopy : What happened in 2024

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Training

2024

- 1-week course on "Méthodes d'étude des Interactions Moléculaires (MedIm) » (Marseille)
- 1-week course on "Molecular Biophysics - Part II" - local PhD students (Grenoble)

WG – Biophysical & Spectroscopy : What's next

Dark blue: Leader platforms
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Updated Nov24

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New EQUIPMENTS

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Updated EQUIPMENTS

- DLS (Montpellier)

FUTURE EQUIPMENTS (in 2025)

- UV module for FIDA (Paris-Saclay)
- AUC-Optima (Grenoble)
- PANTA (Paris-Saclay)

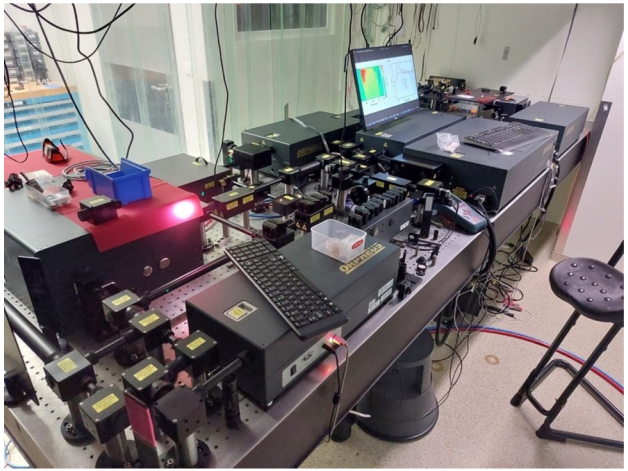
Training

2024

- 1-week course on "Méthodes d'étude des Interactions Moléculaires (MedIm) » (Marseille)
- 1-week course on "Molecular Biophysics - Part II" - local PhD students (Grenoble)

WG - **Biophysical and Spectroscopy : Scientific Highlights**

Femtosecond Stimulated Raman Spectroscopy

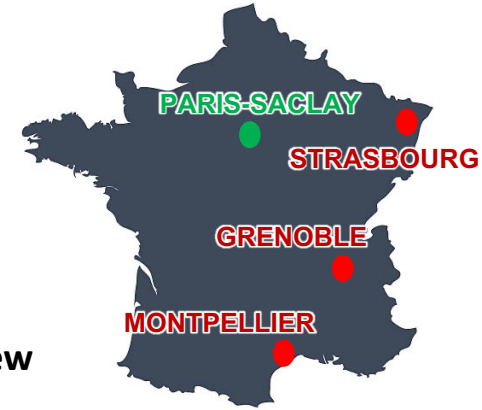


https://www.pepr-luma.fr

Time resolution : 150 fs
Optical range: 315 to 2600 nm
Energy max: 1 mJ /pulse

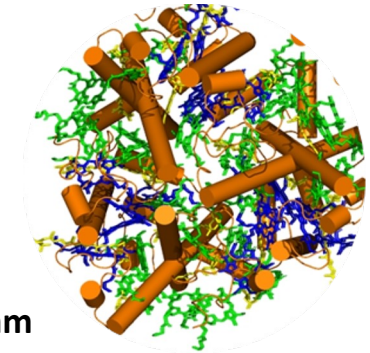
We have designed an apparatus unique in the world capable to tune the Raman pump.

Our set-up allows to yield structural information of the pigment ground and excited states complex natural or artificial systems

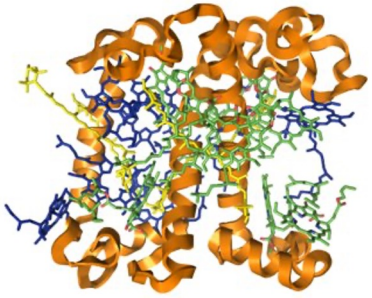


Higher Plants LHCII

upper view



lateral view



24 Chl a
18 Chl b
12 carotenoids

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WG - **Sample Preparation**

Coordinators : A. Poterszman and R. Vincentelli

WG - **Nuclear Magnetic Resonance**

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WG – **Sample Preparation** (coordinators A. Poterszman & R. Vincentelli)

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Cloning and subcloning service					
Multigene construct design					
Bacterial expression					
High Throughput					
Medium scale cultures (up to 6L)					
Large scale cultures (up to 70 L)					
Insect cells expression					
Baculovirus					
Trasient virus free expression					
S2					
Yeast					
Recombinant					
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Large scale cultures					
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Transient expression (hek, cho)					
Vaccinia virus expression					
Large scale cultures					
Cell Free and isotopic labelling					
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Construct screening					
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Small scale paralle/HTP purification					
Preparative Automated purification					

Genotyping					
Development stable cell lines					
Developement of knock-In cell lines *					
Large scale cultures					

Physical access (Training, guidance and assistance to users)
Remote access (Experiments by platform expert and sample send to users)

WG – Sample Preparation : Resource Catalogue

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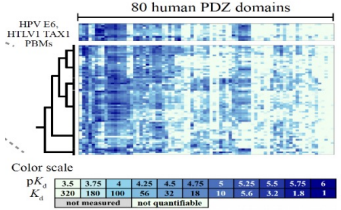
- **HTP bacterial protein production and purification pipelines in the µg/mg scale (Marseille)**

- **HTP Protein-protein or protein-ligand interaction detection and quantification (Marseille)**



- ***Cell Free expression*: proteins and RNA for structural studies (Grenoble)**
- **Wheat germ cell-free expression (Paris-Saclay)**
- **Large scale cultures (Strasbourg)**

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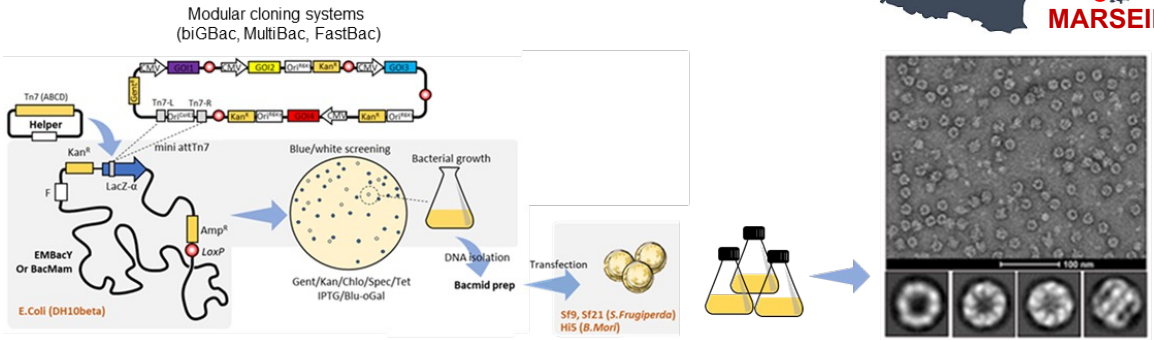
- **Baculovirus single and multigene expression technologies (all centers)**

Bac2Bac, B2F and homologous recombination
Multigene expression systems (MultiBac and BigBac strategies for assembly of multigene expression cassettes)



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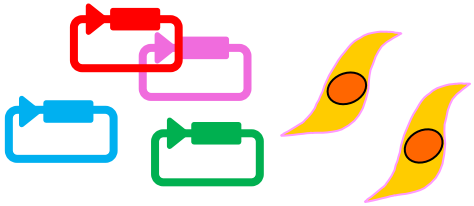
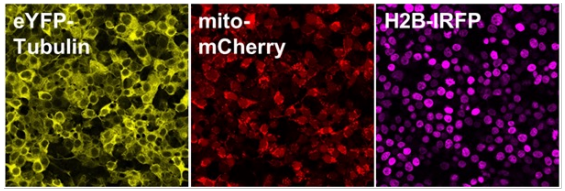
- **Transient expression in mammalian cells (all centers)**

  Transfection in suspension of Mammalian cells (Freestyle, Expi293) with plasmids harboring mammalian promoters (e.g. pcDNA3 with CMV), pTriex/pOPIN

- **Transient expression in insect cells (Strasbourg)**

  Transient transfection in insect cells (Hi5) with plasmids harboring insect promoters





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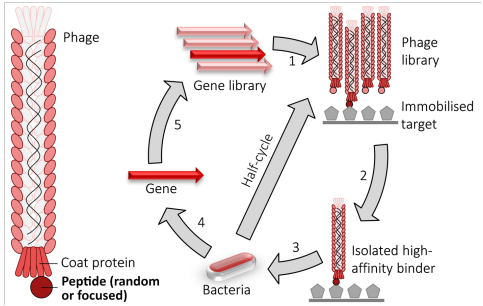
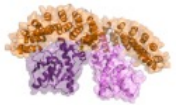
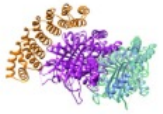
- **Yeast expression of membrane proteins, labelling (Paris-Saclay)**
- **Library-based methods:**
  - **Library screening for bacterial soluble expression (ESPRIT) (Grenoble)**
  - **Nanobody production (Marseille)**
- **Peptide binder phage display (testing as new service-type ; Grenoble)**



- **AlphaRep (Paris Saclay)**



Chevrel A *et al.* J. Struct. Biol. (2018)



Campanacci V *et al. Structure (2019) PNAS (2019); PNAS (2022)*

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- **Yeast expression of membrane proteins, labelling (Paris-Saclay)**
- **Engineering of Mammalian cell lines (Strasbourg, Grenoble)**

  Genome editing and cell line characterization (Strasbourg with TacGene , Grenoble)
  Stable pools (PiggyBac, Sleeping Beauty transposons),
  Large scale cultures

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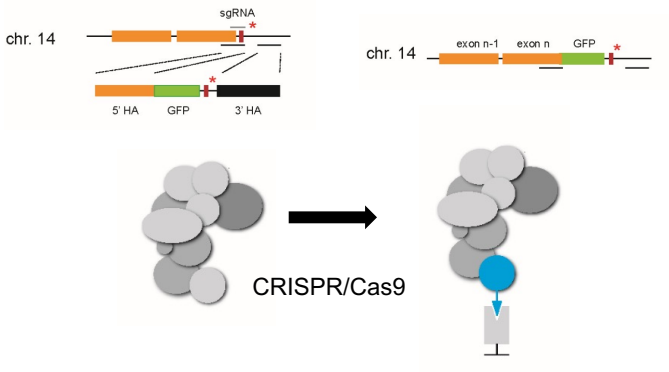
WG – Sample Preparation : Scientific Highlight

Structure of the human TIP60-C histone exchange and acetyltransferase complex

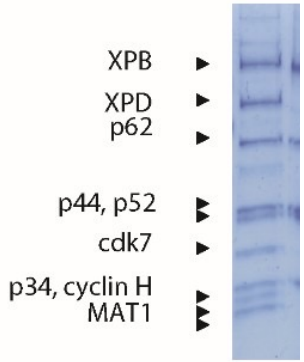
C. Li, E. Smirnova, C. Schnitzler, C. Crucifix, J. P. Concordet, A. Brion, A. Poterszman, P. Schultz, G. Papai & A. Ben-Shem

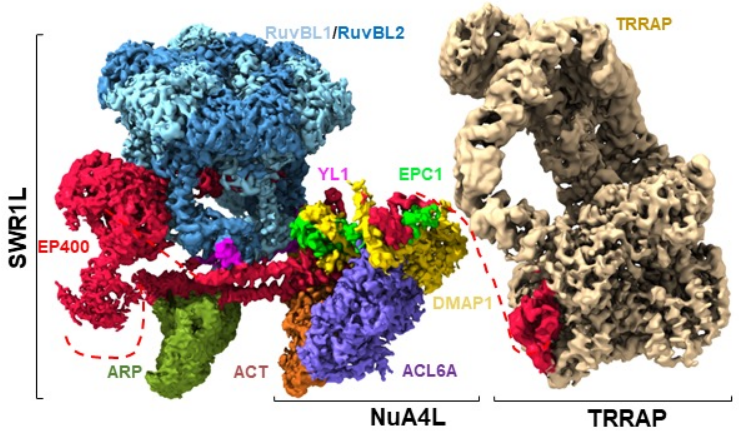
Nature 2024 DOI : 10.1038/s41586-024-08011-w

Endogenous tagging for isolation of protein expressed under physiological conditions



Biomass accumulation for sample preparation in view of structural studies





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WG - **Nuclear Magnetic Resonance**

Coordinator : N. Sibille

WG - **Electron Microscopy**

WG - **Crystallization & X-ray Crystallography**

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WG – **NMR** (coordinator N. Sibille)

- ✓ Access to high-field NMR spectrometers
- ✓ Local experts to supervise/discuss data acquisition & processing

- **Magnets: 600 – 950 MHz**
- **Cryogenic solution NMR probes** (5, 3, and 1.7 mm)
- **^{31}P and ^{19}F optimised solution probes**
- **High-speed MAS probes**
- **HR MAS probes**

- ✓ Funding through FRISBI calls & Infranalytics

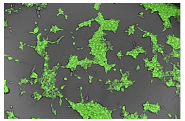
infranalytics

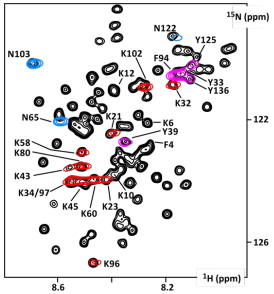
On-going/planned investments:

- ➢ Upgrades of consoles & probes (> 10 years)
- ➢ New probes for specific applications
- ➢ Liquefier / compressors for helium recovery

WG – **NMR : Resource Catalogue**

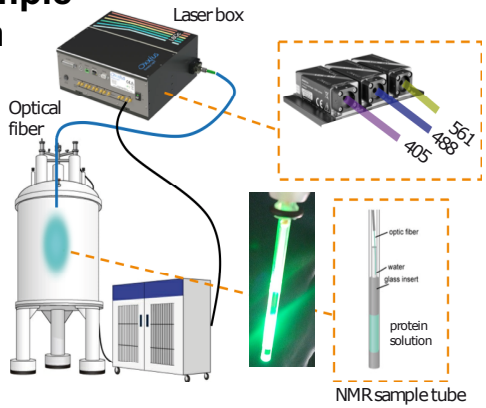
In-cell NMR

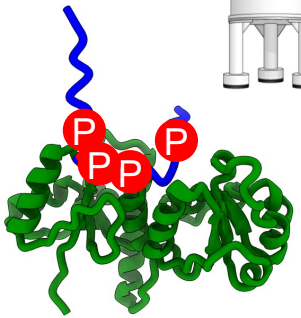




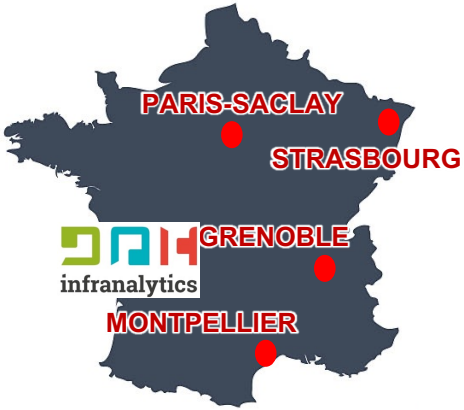
Refrigerated sample changer for metabolomics

In-situ NMR sample illumination





NMR of biomolecules:
IDP, binding, PTM, dynamics
coupling with AlphaFold

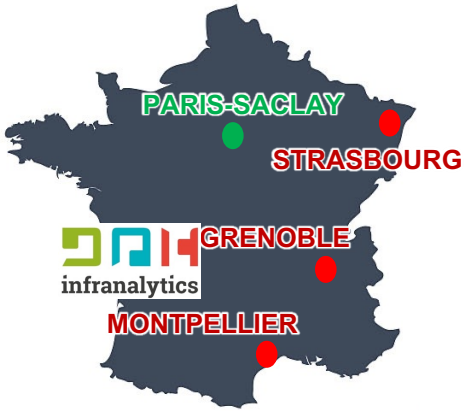


High-pressure NMR

WG – **NMR :** Scientific Highlights (High-pressure NMR)

Daedalus system : ZrO_2 NMR tube + Pump (up to 3000 bar)

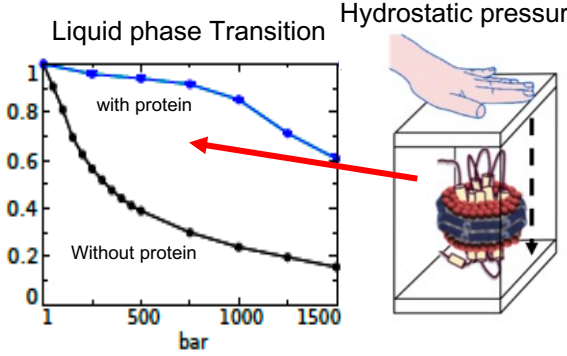
Available for all NMR spectrometers in ICSN site



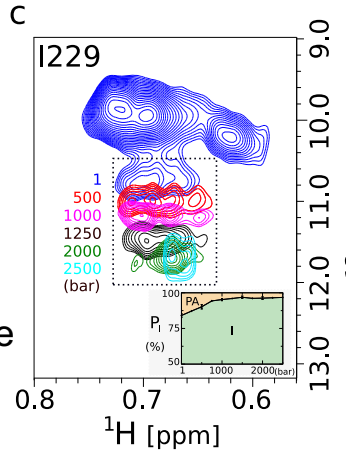
Exploration of the dynamic interplay between lipids and membrane proteins by hydrostatic pressure

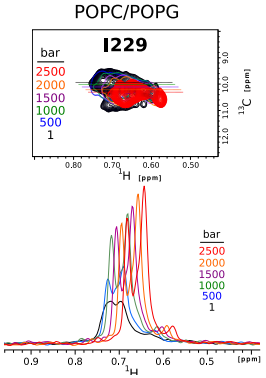
A. Pozza, F. Giraud, Q. Cece, M. Casiraghi, E. Point, M. Damian, C. Le Bon, K. Moncoq, J.-L. Banères, E. Lescop, L. J. Catoire

Nat Commun. 2022 ;13(1):1780. doi: 10.1038/s41467-022-29410-5.



GPCR BLT2 deuterated and specifically isotopic labelled on ^{1}H-^{13}C Ile methyl





FRISBI – **Working Group**

WG - **Biophysical & Spectroscopy**

WG - **Sample Preparation**

WG - **Nuclear Magnetic Resonance**

WG - **Electron Microscopy**

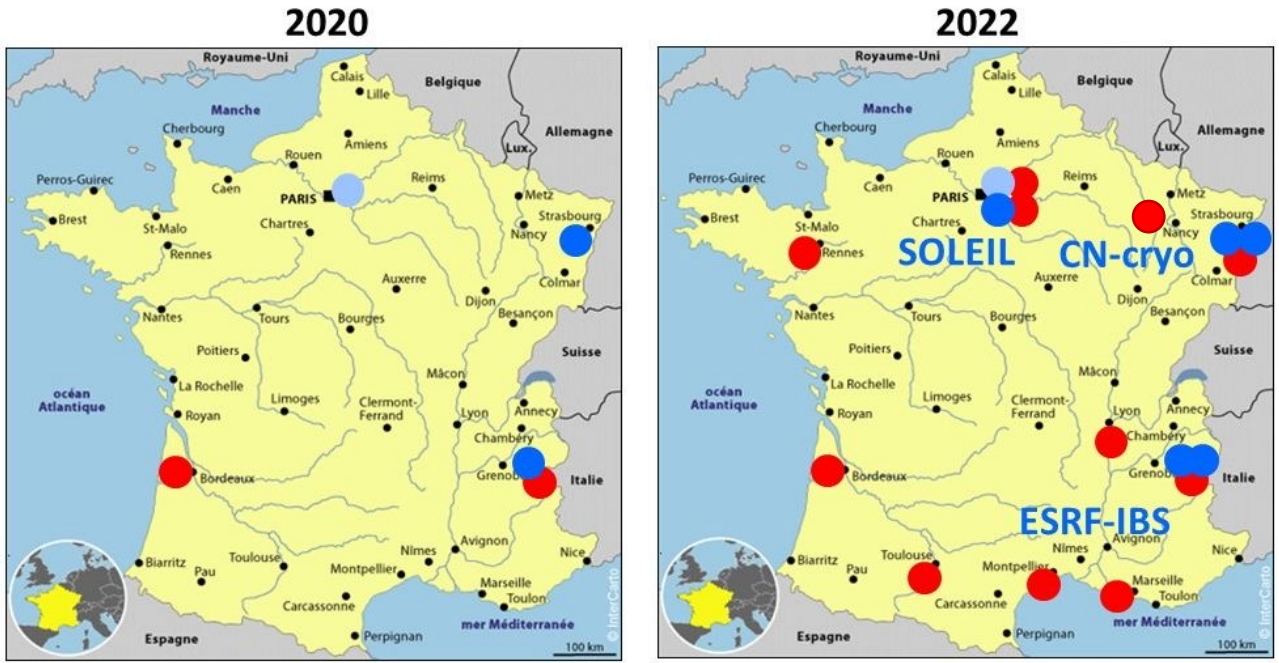
Coordinators : B. Klaholz and G. Schoen

WG - **Crystallization & X-ray Crystallography**

https://frisbi.eu/

WG – **EM** (coordinator B. Klaholz and G. Schoen)

Strategic roadmap for “open-access” cryo-EM in France



- open access high-resolution 300 kV cryo electron microscopes
- Paris-Centre
- new generation 200 kV cryo electron microscopes & high-sensitivity cameras

WG – EM : Resource Catalogue (Projet EquipEx+ France-Cryo-EM)

Installation of 3 Titan Krios G4 instruments at Strasbourg, Grenoble & Paris-Saclay

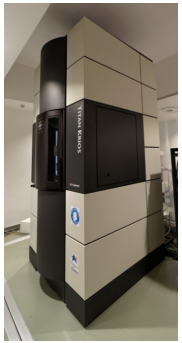
France-Cryo-EM : Krios N°1 - Strasbourg

@CN-cryo, CBI, Illkirch/Strasbourg:

Open to the community since April 2023

FIB / SEM
cryo lamellae

Titan Krios 1
(2013)

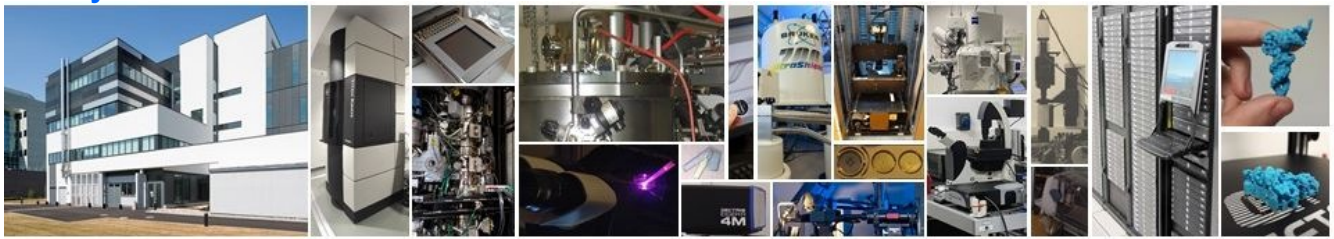


Glacios
(2020)

Titan Krios 2
(2022)

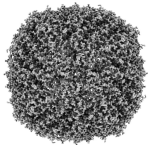
Vitrobot Chameleon Leica GP2

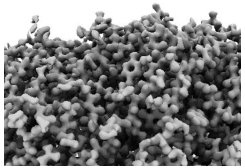
CN-cryo



WG – EM : Resource Catalogue (Projet EquipEx+ France-Cryo-EM)

Installation of 3 Titan Krios G4 instruments at Strasbourg, Grenoble & Paris-Saclay





Contact : cm02.contact@ibs.fr

Pauline Juyoux dans l'audience

France-Cryo-EM : Krios N°2 - Grenoble

Krios CM02 (installed @ESRF managed by IBS/ISBG)

Open to the community January 2025

Cryo-FIB : Aquilos 2

Lift out iFLM

Ouverture aux utilisateurs 2ème – 3ème Trimestre 2025

Glacios 1 / Falcon 4i / K2

Disponible

EM ICE

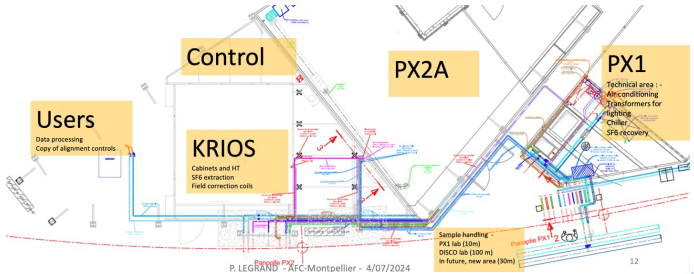
WG – EM : Resource Catalogue (Projet EquipEx+ France-Cryo-EM)

Installation of 3 Titan Krios G4 instruments at Strasbourg, Grenoble & Paris-Saclay

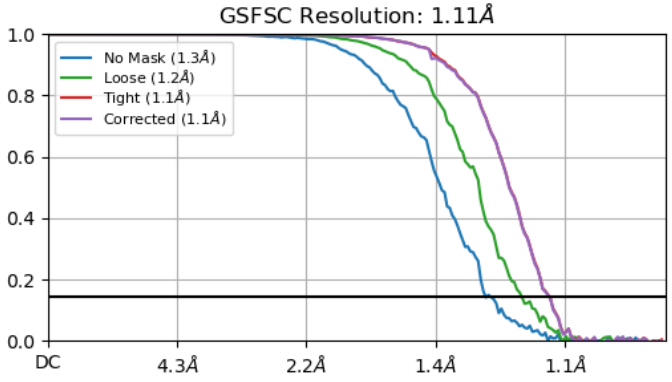
France-Cryo-EM : Krios N°3 - SOLEIL

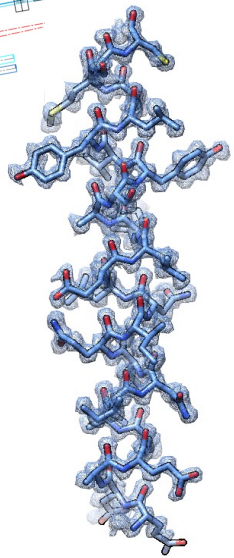
Krios CM03 (installed @SOLEIL

Open to the community November 2024



Fourier Shell Correlation & docking; CWAT 12/23

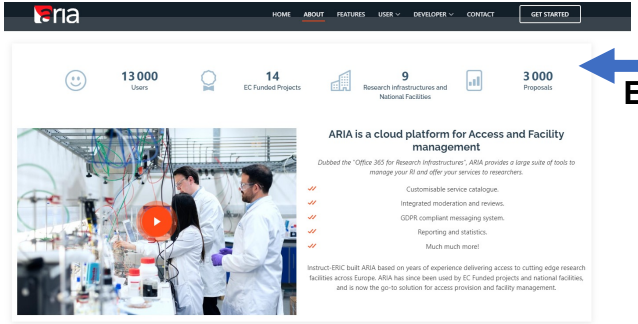




WG – EM : Resource Catalogue (Towards a single access point)

Évaluation de deux mécanismes d'accès (discussions et tests avec les développeurs ARIA et SunSET en cours).
Décision possible a l'automne.

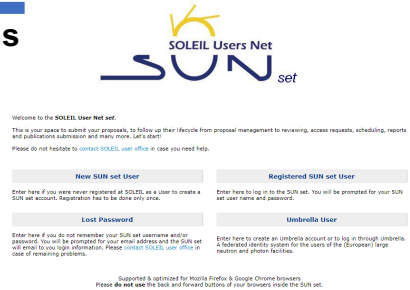
ARIA



projets Européens

Entrée commune

SUNset *(envisagé)*



Outil complet pour la soumission de projets, review par comite indépendant - SOLEIL CP5, biosécurité, planification, reporting, publications.
L'interface Cryo-EM pour l'accès roulant est en beta test.

Utilisé par les lignes de lumière SOLEIL et ESRF CRG (mais pas le CRG Cryo-EM).
Nécessite l'accord de SOLEIL pour l'utiliser.

WG – **EM :** Resource Catalogue (Towards a single access point)

Critères de sélection:

- faisabilité technique & données préliminaires (concentration/distribution de particules, classes 2D, reconstructions 3D, coupes cellulaires, FIB etc.)

- pour particules isolées et / ou tomographie selon les cas

- distribution selon disponibilité ou spécificités techniques de l'instrumentation

Comité de sélection (comité commun):

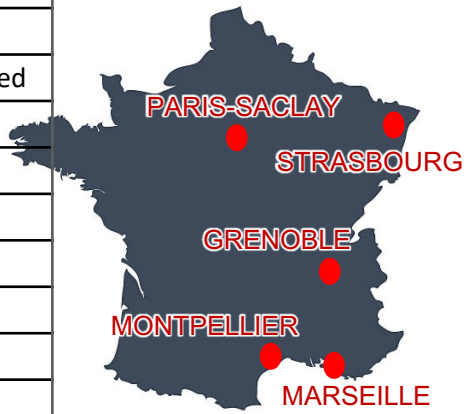
- comité composé des membres FRISBI existants + scientifiques nationaux

Plan de gestion des données

- soumis

WG – EM : Resource Catalogue (Cryo-EM deployment 2024)

Site	Instrumentation	State
IGBMC, Ilkirch	Titan Krios 300 kV I & II, Glacios 200 kV, Chameleon	Running
	Freezing devices / plasma cryo-FIB	Tender / Funding pending
IBS/ESRF, Grenoble	Glacios 200 kV / Titan Krios 300 kV	Running / Final phase validated
	Glacios Camera upgrade / cryo-FIB	Ordered
SOLEIL, St-Aubin	Titan Krios 300 kV	Running
Pasteur Institute, Paris	Titan Krios 300 kV, Glacios 200 kV x2, cryo-FIB	Running
	BSL2 plasma cryo-FIB, BLS3 cryo-TEM & cryo-CLEM plasma FIB	Operationnal by 2028
Curie Institute, Paris	Glacios 2 200 kV / High speed high pressure freezer	Running
	cryo & RT CLEM	Funding pending
Univ Paris-Cité	cryo-EM 200 kV	Tender 2024 ?
I2BC, Saclay	Glacios 2 200 kV	Installed
CBI, Toulouse	Talos Artica 200 kV / cryo-FIB-SEM	Running / Funding pending
IECB, Bordeaux	Talos Artica 200 kV / Glacios 2 200 kV	Running / Installed
IGDR, Rennes	CRYO ARM 200 kV	Commissioning June 2024
IMoPA, Nancy	Glacios 2 200 kV	Installed Nov 2023
ENS, Lyon	cryo-EM 200 kV	Tender pending co-funding
AFMB, Marseille	Glacios 2 200 kV	Installed
CBS, Montpellier	High pressure freezer / cryo-FIB-SEM / 200 kV planned	Tenders 2023 & 2024 / planned



based on roadmap established in 2016 & initial France-Cryo-EM project

FRISBI – **Working Group**

WG - **Biophysical & Spectroscopy**

WG - **Sample Preparation**

WG - **Nuclear Magnetic Resonance**

WG - **Electron Microscopy**

WG - **Crystallization & X-ray Crystallography**

Coordinators : A. Mc Ewen, G. Sulzenbacher and S. Morera

https://frisbi.eu/

WG – **Crystallization & X-ray crystallography :**

Dark blue: National entry point per technology
Light blue: Technology available

Methods/Nodes	Grenoble	Marseille	Montpellier	Paris-Saclay	Strasbourg
Automatic pipetting					
96-plate well dispensing					
Nanoliter pipetting					
Manual refinement	advices to users				
Automated refinement screen pipetting					
Crystal growth					
4 °C					
8 °C					
18 °C					
20 °C					
28 °C					
Controlled atmosphere					
Microfluidics					
Crystal handling					
Manual					
Automated harvesting					
Controlled atmosphere					
X-ray diffraction					
Inhouse X-ray source					
Automated beamline	ESRF				
Cryo system	ESRF				
In situ diffraction	ESRF				
BAG Access	Instruct, Isidore, …				
Co-crystalllization					
Nanobodies	users bring their nanobodies				
Complexes					
rMMS	users bring seeds				
Alpha-repeats					
XO4	users bring XO4				
Membrane protein crystallization					
Cubic phase					
FRAP					
Ligand screening					
Fragment screening					
Optimization of binders					
In-situ chemistry					

IGBMC, Strasbourg
Alastair McEWEN
Pierre POUSSIN-COURMONTAGNE

I2BC, Paris-Saclay
Solange MORERA
Armelle VIGOUROUX

CBS, Montpellier
Vanessa DELFOSSE
François HOH
Muriel GELIN

IBS, Grenoble
Josan MARQUEZ
Florine DUPEUX

AFMB, Marseille
Gerlind SULZENBACHER
Véronique ROIG-ZAMBONI

WG – Crystallization & X-ray crystallography : Resource Catalogue

Dark blue: National entry point per technology
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Complexes					
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Alpha-repeats					
XO4	users bring XO4				
Membrane protein crystallization					
Cubic phase					
FRAP					
Ligand screening					
Fragment screening					
Optimization of binders					
In-situ chemistry					

- **Controlled atmosphere in crystal growth and Cubic-phase crystallization (Grenoble)**
- **Nanobodies production to facilitates crystal growth (Marseille)**
- **Ligand screening, mini fragments and click chemistry (Montpellier)**
- **Alpha-repeats production and Fluorescence recovery after photobleaching (FRAP)**

WG – Crystallization & X-ray crystallography : What happened in 2024

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XO4	users bring XO4				
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Cubic phase					
FRAP					
Ligand screening					
Fragment screening					
Optimization of binders					
In-situ chemistry					

Equipment acquisition

New EQUIPMENTS

- RockImager 360UV (Strasbourg)

Training

2024

- FRISBI members continue to be very active in ЯÉCIPROCS-bio
- ЯÉCIPROCS-bio, Micro-ED school, Montpellier, June 2024
- ЯÉCIPROCS-bio, TEA-BAG, User meeting Soleil 2024
- ЯÉCIPROCS-bio meeting 13/10/2024

WG – Crystallization & X-ray crystallography : What's next

Dark blue: National entry point per technology
Light blue: Technology available

Methods/Nodes	Grenoble	Marseille	Montpellier	Paris-Saclay	Strasbourg
Automatic pipetting					
96-plate well dispensing					
Nanoliter pipetting					
Manual refinement	advices to users				
Automated refinement screen pipetting					
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8 °C					
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Microfluidics					
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Inhouse X-ray source					
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Cryo system	ESRF				
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FRAP					
Ligand screening					
Fragment screening					
Optimization of binders					
In-situ chemistry					

Equipment acquisition

New EQUIPMENTS

- RockImager 360UV (Strasbourg)

FUTURE EQUIPMENTS (in 2025)

- Mosquito upgrade (Marseille)
- RockImager 360 UV (Marseille, Montpellier) ???

Grouped purchase of RockImagers might be consider to obtain a good deal

Training

2024

- FRISBI members continue to be very active in ЯÉCIPROCS-bio
- ЯÉCIPROCS-bio, Micro-ED school, Montpellier, June 2024
- ЯÉCIPROCS-bio, TEA-BAG, User meeting Soleil 2024
- ЯÉCIPROCS-bio meeting 13/10/2024

In 2025 (September)

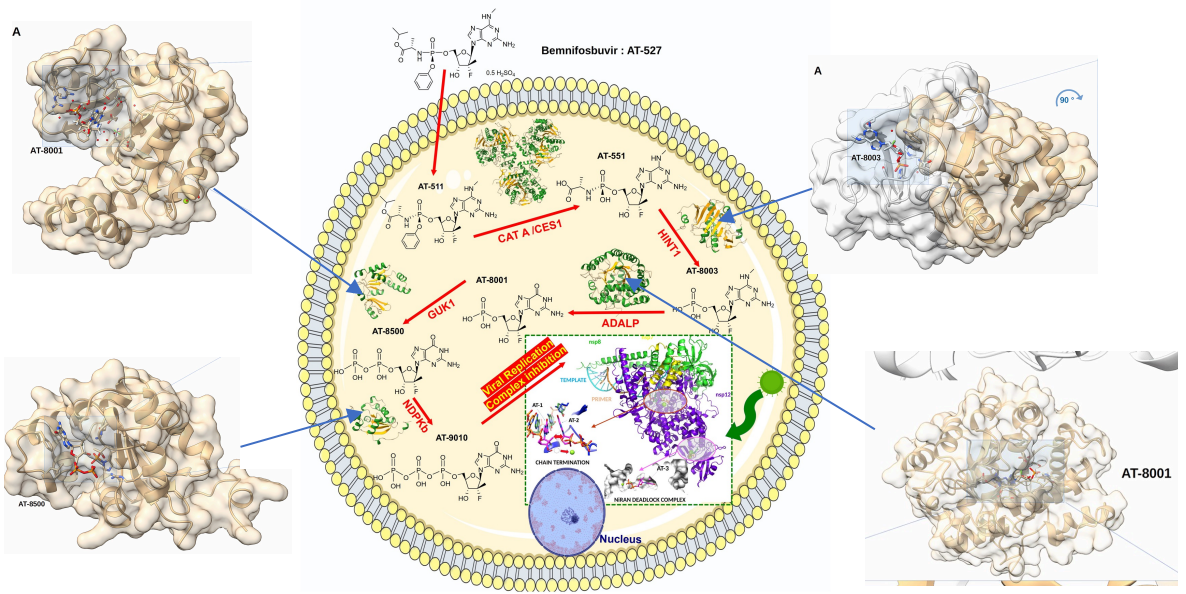
- ANF (Action nationale de Formation) in preparation for 2025, SOLEIL - by ЯÉCIPROCS-bio

The activation cascade of the broad-spectrum antiviral bemnifosbuvir characterized at atomic resolution.

Aurélie Chazot, Claire Zimberger, Mikael Feracci, Adel Moussa, Steven Good, Jean-Pierre Sommadossi, Karine Alvarez, François Ferron et Bruno Canard.

PLOS Biology, 2024.

DOI : https://doi.org/10.1371/journal.pbio.3002743



FRISBI – User Commitee

WG - Biophysical & Spectroscopy

Stéphane BETZI *(CRCM, Marseille)*
Eric ENNIFAR *(IBMC, Strasbourg)*

WG - Sample Preparation

Paola LLINAS *(i2BC, Paris-Saclay)*
Valérie BIOU *(IBPC, Paris)*

WG - Nuclear Magnetic Resonance

Rémy SOUNIER *(IGF, Montpellier)*
Guillaume BOUVIGNIES *(ENS, Paris)*

WG - Electron Microscopy

Vincent CHAPTAL *(MMSB, Lyon)*
Artemis KOSTA *(IMM, Marseille)*

WG - Crystallization & X-ray Crystallography

Louis RENAULT *(i2BC, Paris-Saclay)*
Claude SAUTER *(IBMC, Strasbourg)*