

# French Infrastructure for Integrated Structural Biology



**FRISBI User Meeting**

*Gif sur Yvette*

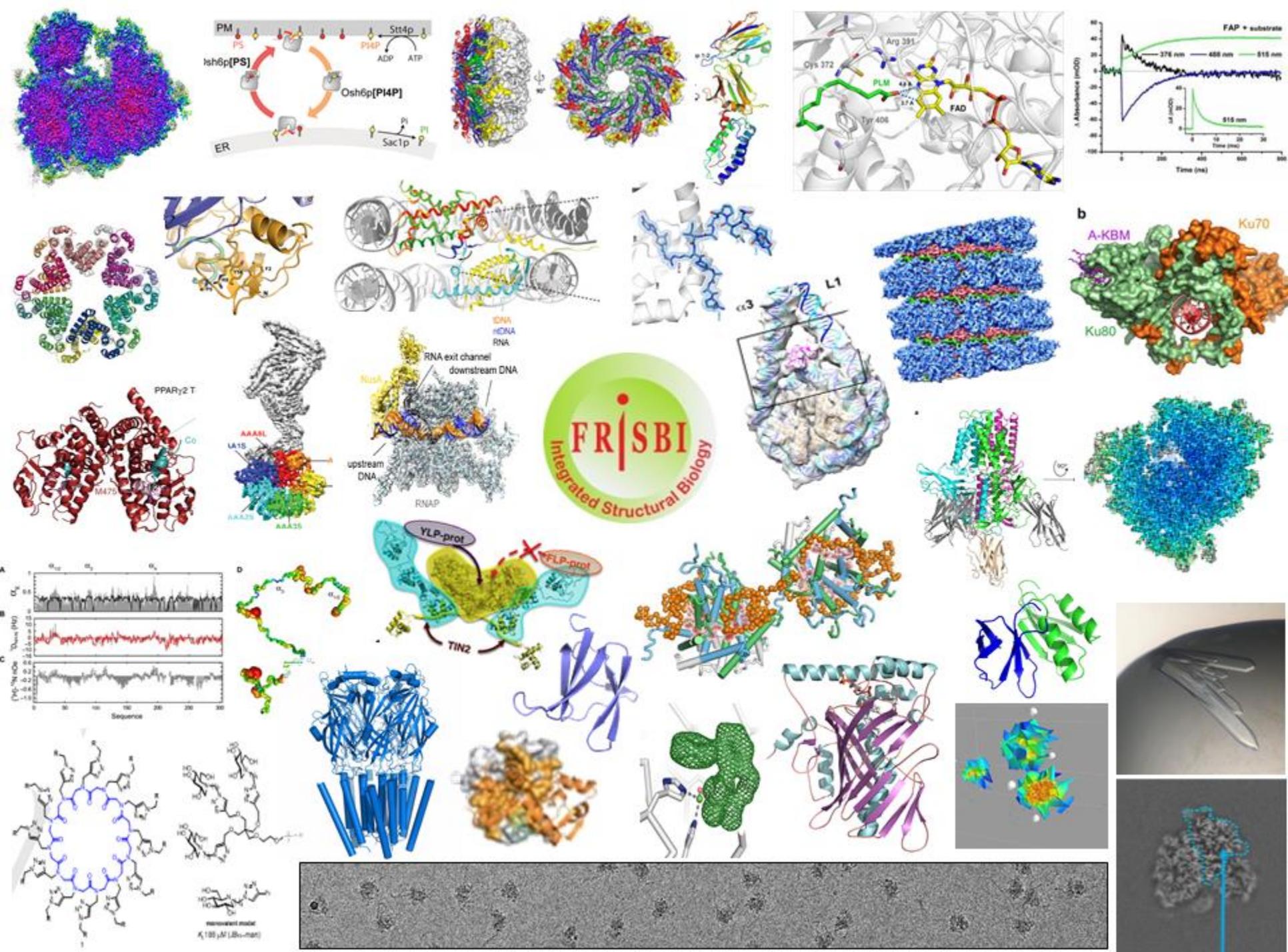
**January 17<sup>th</sup> 2024**



# French Infrastructure for Integrated Structural Biology

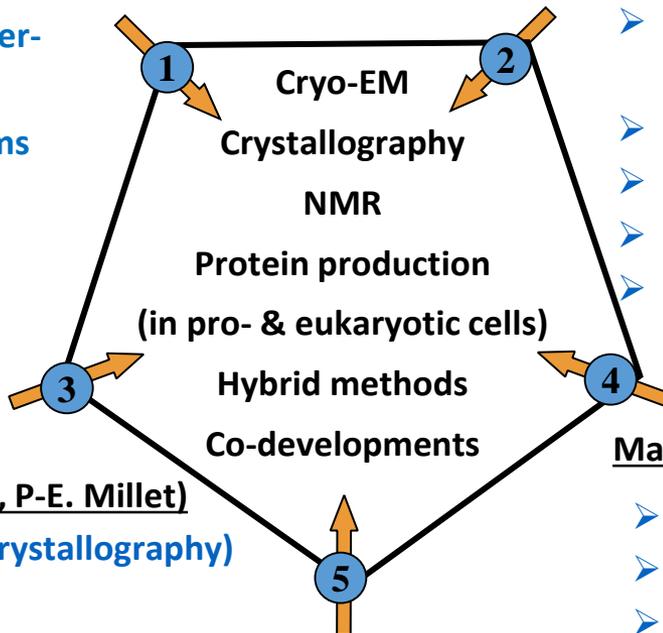


5 FRISBI centres (Strasbourg, Grenoble, Montpellier, Marseille, Paris-Saclay)  
Strasbourg and Grenoble are Instruct-ERIC centres



## Strasbourg (B. Klaholz, A. Poterszman)

- Cryo-EM & tomography, super-resolution microscopy
- Eukaryotic expression systems
- Correlative microscopy
- Crystallography
- Multi-resolution integration



## Montpellier (M. Cohen-Gonsaud, P-E. Millet)

- Small molecules screening (crystallography)
- SAXS
- High-pressure spectroscopy
- $\mu$ ED

## Grenoble (D. Hart, M. Ringkjøbing Jensen)

- Cryo-EM, crystallography developments (with ESRF)
- NMR, Membrane proteins
- Biophysics & sample QC
- Expression (cell-free & soluble fragments)
- Automated ligand screening (EMBL)

## Marseille (Y. Bourne, G. Sulzenbacher)

- Nanobodies
- HTP protein production & interactions
- Eukaryotic expression systems

## Paris-Saclay (J. Ménétreay, S. Zinn)

- Biophysics
- Crystallography, NMR, Cryo-EM
- SwitchSense, Alpha-Rep
- Eukaryotic expression systems
- Synchrotron (SOLEIL)

❖ *Complementary node expertise*

❖ *Transversal working groups*

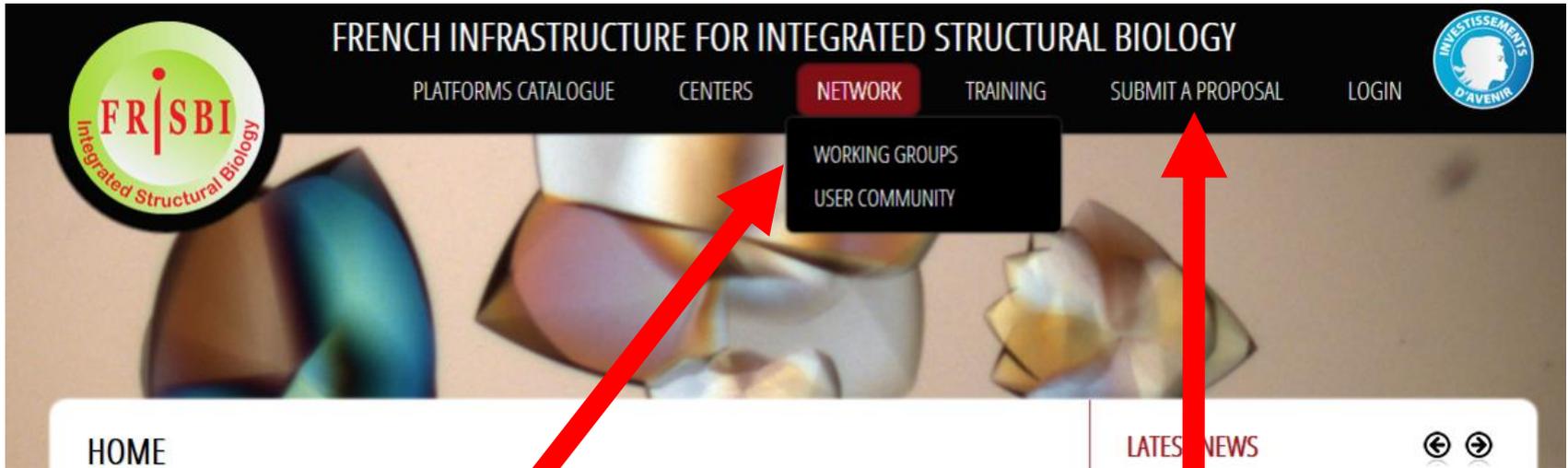


# One portal for access

Web site: accessible via the Instruct, CNRS, ReNaFoBis & ITMO websites

<https://frisbi.eu>

[contact@frisbi.eu](mailto:contact@frisbi.eu)



**Working group and FRISBI user community**

**Submit a proposal**

**150 projects** through specific calls for funded access

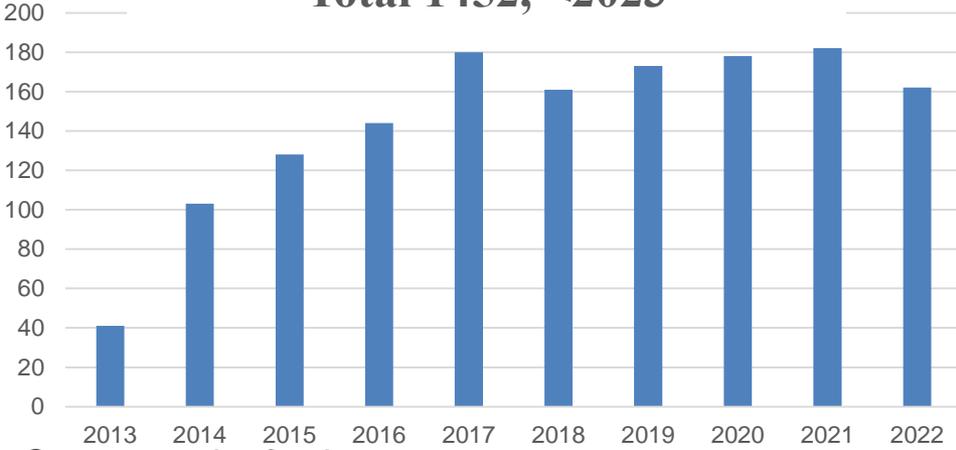
Specific calls for transversal organization of training workshops

2022, call for **inter-infra projects** (with FBI & ProFI) co-funded by IBiSA

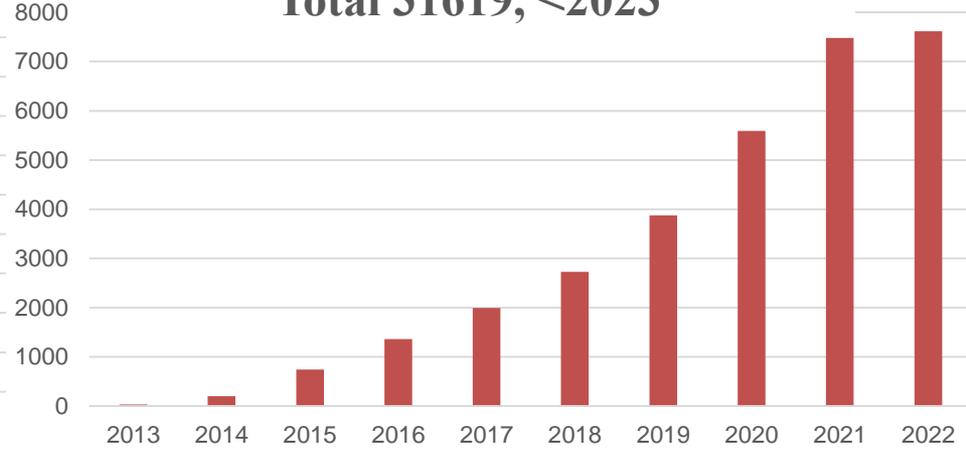


# Scientific Impact

### Publications mentioning FRISBI Total 1452, <2023



### Articles citing FRISBI publications Total 31619, <2023



Source: web of science

## 277 high visibility publications citing FRISBI IF>6 (<2022)

- 10 *Nature*
- 9 *Science*
- 3 *Cell*
- 27 *JACS*
- 10 *Mol Cell*
- 6 *NSMB*
- 55 *Nature Communications*

# Metrics of the FRISBI infrastructure

- user projects: 258 (2012) → 1276 (2020), > 10 500 in total (end of 2022)
- users trained on FRISBI platforms: > 250 / year, > 3 000 in total (end of 2022)
- specialized workshops: ~ 8 / year

- national school:



**11<sup>th</sup> edition of ReNaFoBiS summer school,  
May 30 - June 7th 2024, Oléron**

# General Perspectives

- Continue to serve the community:
  - access
  - training
- Participate in European infrastructures (**Instruct-ERIC, iNEXT-Discovery**)
- Continue **interactions** with other national (**FBI, IFB, PROFI**) infrastructures, e.g. inter-infra calls (IBiSA-funded and as part of "Club INSB inter-infrastructures" + data centres etc.)
- Be part of the ongoing road map of infrastructures (ministry of research)
- Continue to develop **partnerships** with National & European companies (working group)
- Develop **new approaches** & **develop integrated approaches** at interface of **molecular & cellular structure/function** (cryo-EM, CLEM, FIB-tomo, sample preparation, micro electron diffraction, in-cell NMR and EPR, fragment screening)
  - **international visibility, integrated structural (cellular) biology, molecular mechanisms & therapeutic targets**

# 2024 plans

- **User meeting / survey essential for support from the community**
- **Scientific advisory board (SAB)**
- **Instrumentation (France 2030 call, submitted)**
- **PIA2 (FRISBI-2) ends → international evaluation April 2024**
- **Long-term sustainability (Ministry of Research, submitted)**  
*running costs, maintenance, R&D, support for user access*
- **Extension to comprise « plateformes labellisées FRISBI »; call planned 2025**  
**Some criteria: service activity for internal and external users (expertise + admin support); strong scientific environment; R & D; training etc.**



# FRISBI working groups



# Protein expression



# FRISBI resources for sample preparation

**Grenoble**

**Marseille**

**Montpellier**

**Paris-Saclay**

**Strasbourg**

	Grenoble	Marseille	Paris-Saclay	Strasbourg	Montpellier
<b>Sample Preparation</b>					
<b>Cloning</b>					
Cloning and subcloning service					
Multigene construct design					
<b>Bacterial expression</b>					
High Throughput					
Medium scale cultures (up to 6L)					
Large scale cultures (up to 70 L)					
<b>Insect cells expression</b>					
Baculovirus					
Transient virus free expression					
S2					
<b>Yeast</b>					
Recombinant					
Medium scale cultures					
Large scale cultures					
<b>Mammalian</b>					
Transient expression (hek, cho)					
Vaccinia virus expression					
Else					
Large scale cultures					
<b>Cell Free and isotopic labelling</b>					
Cell free production					
Labelling					
<b>Library based methods</b>					
Construct screening					
Selection of binders (Nanobodies)					
<b>Protein purification</b>					
Medium scale purification					
Small scale parallel/HTP purification					
Preparative Automated purification					
<b>Genotyping</b>					
Development stable cell lines					
Development of knock-in cell lines *					
Large scale cultures					

## Bacterial and cell free expression

- HTP bacterial protein production and purification pipelines in the  $\mu\text{g}/\text{mg}$  scale (Marseille)
- HTP Protein-protein interaction detection and quantification (Marseille)
- Cell Free expression: proteins and RNA for structural studies (Grenoble)
- Large scale cultures (Strasbourg)

## Insect cell and Mammalian expression

- Baculovirus single and multigene expression technologies (all centers)
- Transient expression in mammalian cells (all centers)
- Transient expression in insect cells (Strasbourg)

- Bac2Bac, B2F and homologous recombination
- Multigene expression systems (MultiBac and BigBac strategies for assembly of multigene expression cassettes)
- Transient transfection in Mammalian cells (Freestyle, Expi293) with plasmids harboring mammalian promoters (e.g. pcDNA3 with CMV), pTriex/pOPIN
- Transient transfection in insect cells (Hi5) with plasmids harboring insect promoters (e.g. pOpIE2)

Physical access (Training, guidance and assistance to users)

Remote access (Experiments by platform expert and sample send to users)



# FRISBI resources for sample preparation

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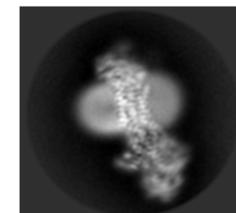
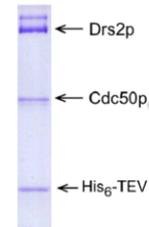
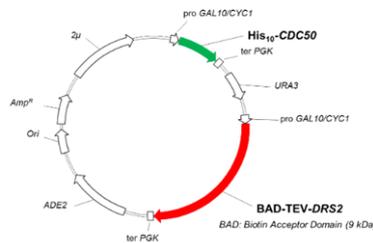
**Yeast expression**  
 Yeast expression of membrane proteins, labelling (Paris-Saclay)

## Engineering of Mammalian cell lines

Genome editing and cell line characterization (Strasbourg with TacGene , Grenoble)  
 Large scale cultures

## Library-based methods

Nanobody production (Marseille)  
 Library screening for bacterial soluble expression (ESPRIT) (Grenoble)  
 Peptide binder phage display (testing as new service-type; Grenoble)



Grenoble

Marseille

Montpellier

Paris-Saclay

Strasbourg

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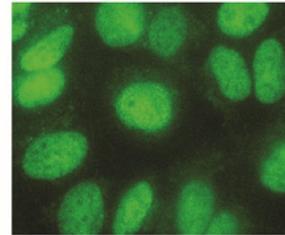
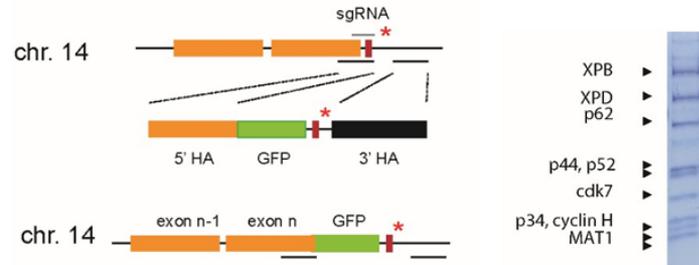
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# FRISBI resource catalogue for sample preparation

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Marseille

Montpellier

Paris-Saclay

Strasbourg

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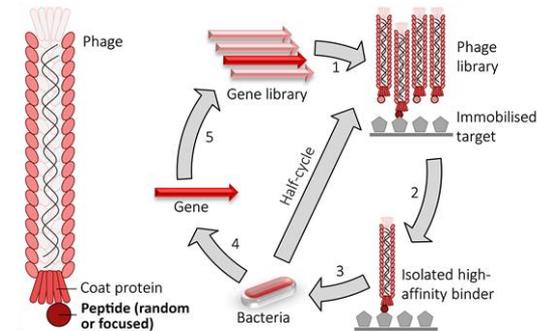
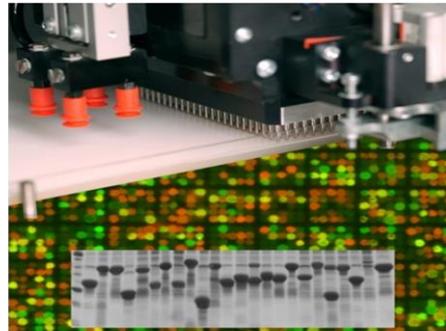
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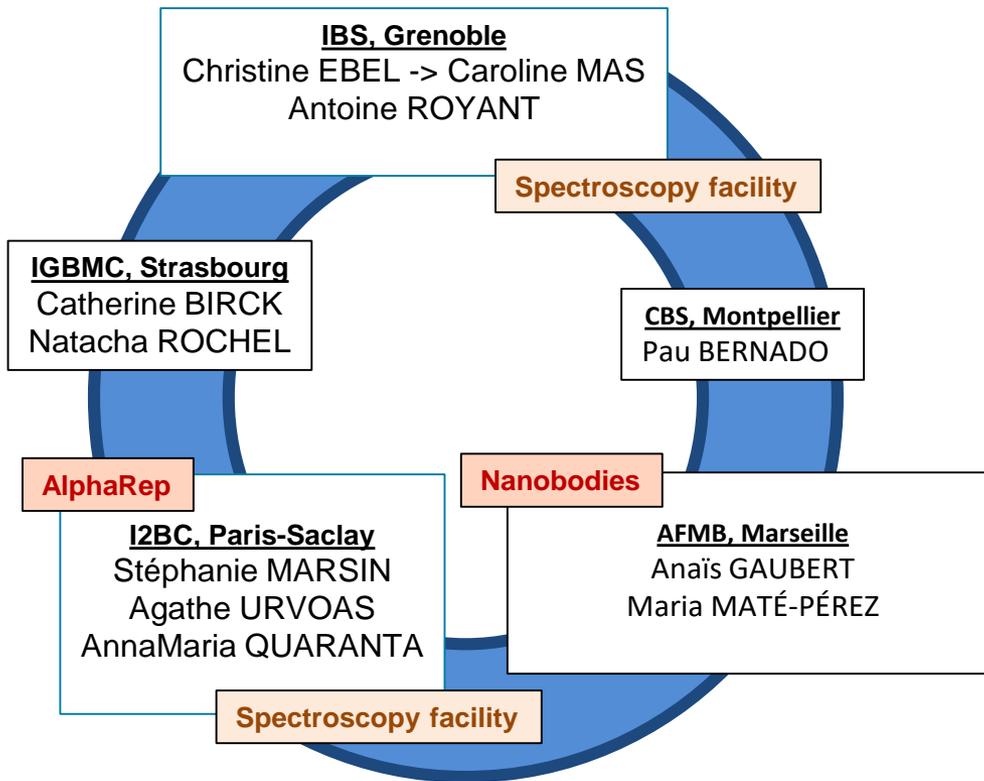


Physical access (Training, guidance and assistance to users)



# Biophysical characterization

# WG - Biophysical and Spectroscopy (C. Ebel and J. Ménétrey)



Technology available Leader Platforms	Grenoble	Marseille	Paris-Saclay	Strasbourg	Montpellier
Updated JAN24					
<b>Structure &amp; Oligomers</b>					
CD					
SEC-MALLS					
Anal Ultracent					
Automated PEAQ DSC					
FSEC					
NanoDSF					
Mass Photometry			2024		
<b>Protein Interactions &amp; mobility</b>					
ITC					
SPR					
Bio-Layer Interferometry					
Microscale Thermophoresis					
FIDA			2024		
SwitchSense /Helix					
<b>Spectroscopies</b>					
EPR					
EPR high field					
FTIR					
RAMAN					
Super resolution microscopy					
Electronic Spectroscopy					
<i>In crystallo</i> optical spectroscopy	ic05 Lab				
<b>SAXS in house</b>					
SAXS in house					
<b>Binders</b>					
Nanobodies					
Alpha-Rep					

# WG - Biophysical and Spectroscopy

## what happened in 2023 and what's next in 2024



### Equipment acquisition

#### New EQUIPMENTS

- PEAQ-ITC from Malvern-Panalytical (Strasbourg)
- Mass photometry (Paris-Saclay)

#### FUTURE EQUIPMENTS (in 2024)

- Mass photometry (Marseille and Strasbourg)

- AUC-Optima (Grenoble)

#### Updated EQUIPMENTS

- OMNISEC (Grenoble)
- Switch Sense -> heliX+ (Paris-Saclay)
- ITC 200 (Strasbourg)
- Octet R8 (Marseille)

### Coordination of Training

#### 2023

- 1-week course on "Molecular Biophysics - Part I" - local PhD students (Grenoble)
- 2023 - **EMBO practical course** "Preparation and biophysical/MS characterization of multiprotein complexes for cryo-EM analysis" (Strasbourg)

#### 2024

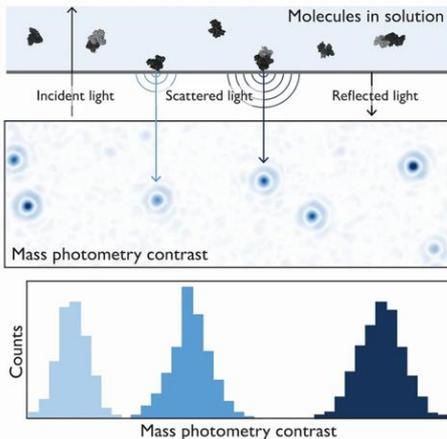
- 1-week course on "Méthodes d'étude des Interactions Moléculaires (MedIm)"
- 1-week course on "Molecular Biophysics - Part II" - local PhD students (Grenoble)

# Mass Photometry

- Available at Grenoble and Paris-Saclay node
- Acquisition in 2024 for Strasbourg node



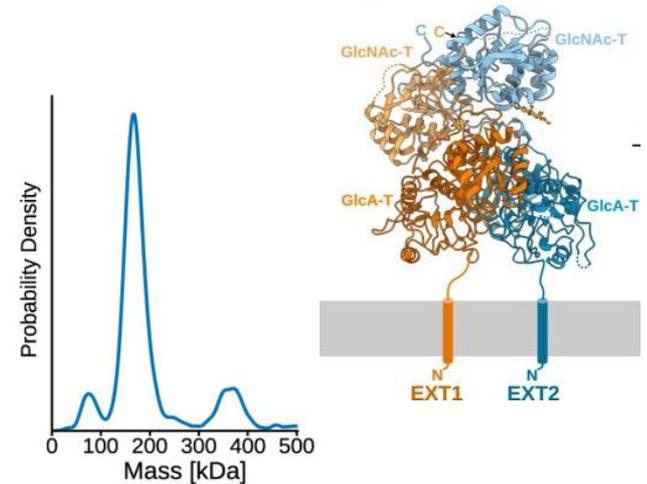
RE•FEYN



**Mass photometry is a novel bioanalytical technology that measures molecular mass by quantifying light scattering from individual biomolecules in solution**

- Fast and easy experiment
  - Time : 1 min
  - Sample : 20 ul @ 10 nM
- Suitable for membrane proteins analysis in presence of detergents

**Structure of the human heparan sulfate polymerase complex EXT1-EXT2**  
Leisico et al (2022) Nature Com  
IBS Grenoble, EMBL Grenoble



From mass photometry, co-expression of EXT1 or EXT2 leads to a majority of dimer

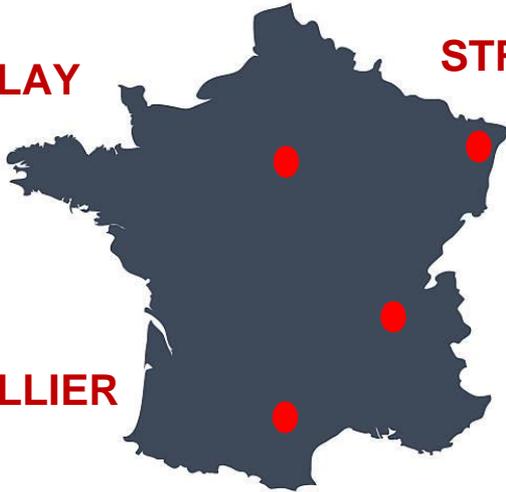


**NMR**

PARIS-SACLAY



STRASBOURG

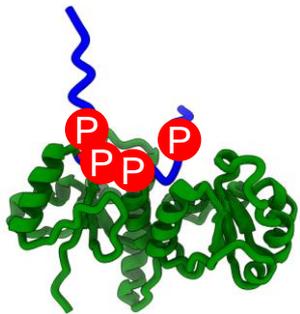


GRENOBLE



MONTPELLIER

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

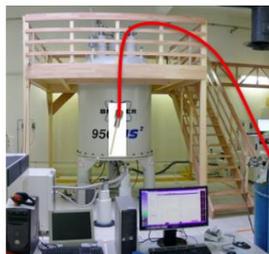


*NMR of biomolecules:  
IDP, binding, PTM, dynamics  
Coupling with AlphaFold*

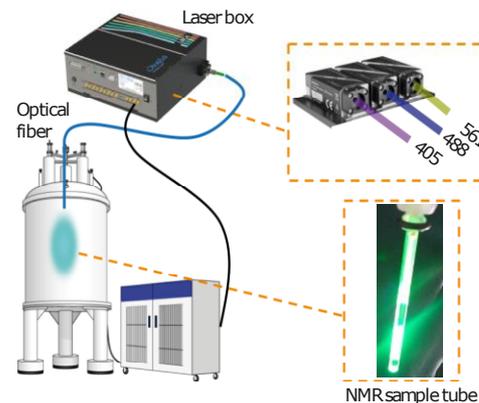
*Refrigerated  
sample changer  
for metabolomics*



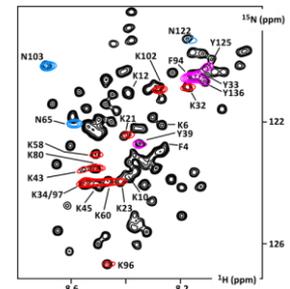
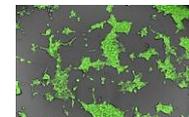
*High-pressure NMR*



*In situ NMR sample  
illumination*



*In cell NMR*





- ✓ Access to high-field NMR spectrometers
- ✓ Local experts to supervise/discuss data acquisition & processing
- ✓ Funding through FRISBI calls & Infranalytics



### On-going/planned investments:

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





# X-ray crystallography

# Working Group Crystallization & X-ray *Crystallography*



## **Contacts**

### **Grenoble:**

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Florine Dupeux, [florine.dupeux@ibs.fr](mailto:florine.dupeux@ibs.fr)

### **Marseille:**

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Véronique Roig-Zamboni, [veronique.zamboni@univ-amu.fr](mailto:veronique.zamboni@univ-amu.fr)

### **Montpellier:**

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Vanessa Delfosse, [vanessa.delfosse@cbs.cnrs.fr](mailto:vanessa.delfosse@cbs.cnrs.fr)

### **Paris-Saclay:**

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Armelle Vigouroux, [armelle.vigouroux@i2bc.paris-saclay.fr](mailto:armelle.vigouroux@i2bc.paris-saclay.fr)

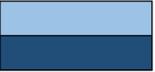
### **Strasbourg:**

Alastair McEwen, [alastair@igbmc.fr](mailto:alastair@igbmc.fr)

Pierre Poussin-Courmontagne, [poussin@igbmc.fr](mailto:poussin@igbmc.fr)

# Services to Users (Academic and industrial)

Methods/Nodes	Grenoble	Marseille	Montpellier	Paris-Saclay	Strasbourg
<b>Automatic pipetting</b>					
96-plate well dispensing	Technology available				
Nanoliter pipetting	Technology available				
Manual refinement	Technology available				
<b>Crystal growth</b>					
4 °C	Technology available				
8 °C	Technology available				
18 °C	Technology available				
20 °C	Technology available				
28 °C	Technology available				
Controlled atmosphere	Leader platform	Technology available	Technology available	Technology available	Technology available
Microfluidics	Technology available				
<b>Crystal handling</b>					
Manual	Technology available				
Crystal direct	Leader platform	Technology available	Technology available	Technology available	Technology available
Controlled atmosphere	Leader platform	Technology available	Technology available	Technology available	Technology available
<b>X-ray diffraction</b>					
Inhouse X-ray source	Technology available				
Cryo system	Technology available				
In situ diffraction	Technology available				
BAG Access	Technology available				
<b>Co-crystallization</b>					
Nanobodies	Technology available	Leader platform	Technology available	Technology available	Technology available
Complexes	Technology available				
Alpha-repeats	Technology available	Technology available	Technology available	Leader platform	Technology available
<b>Membrane protein crystallization</b>					
Cubic phase	Leader platform	Technology available	Technology available	Technology available	Technology available
FRAP	Technology available	Technology available	Technology available	Leader platform	Technology available
<b>Ligand screening</b>					
Fragment screening	Technology available	Technology available	Leader platform	Technology available	Technology available
Optimization of binders	Technology available	Technology available	Leader platform	Technology available	Technology available
In-situ chemisrty	Technology available	Technology available	Leader platform	Technology available	Technology available


  
 Technology available
   
 Leader platform

[https://frisbi.eu/  
platforms-catalogue/](https://frisbi.eu/platforms-catalogue/)

# Investments 2023 (funded by FRISBI or others)

## New EQUIPMENTS

Formulatrix Rock Imager 360 UV (co-funded by Inserm and Région Grand Est), Strasbourg  
Dragonfly (SPT Labtech, IBISA funding), Montpellier  
Labctye ECHO acoustic Dispenser, Grenoble

## Future EQUIPMENTS

Formulatrix RI1000 (most likely second hand), Paris-Saclay

## Coordination of Training and Meetings

### Organization

ЯÉCIPROCS satellite meeting to BSI3, Marseille  
ЯÉCIPROCS –bio, Micro-ED 2 users), Montpellier

### Future

TEA-BAG, User meeting Soleil 2024, Paris-Saclay  
ANF (Action nationale de Formation) in preparation for 2025 (5 days), Paris-

Saclay

Micro-ED 2024, Montpellier



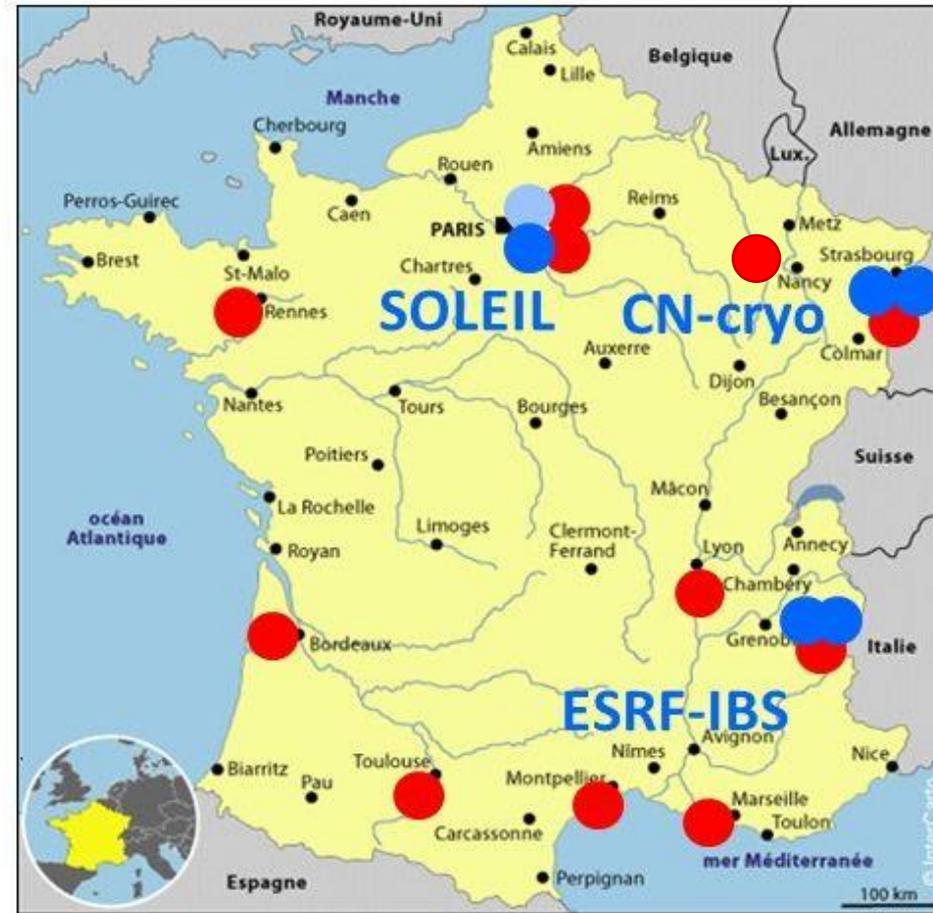
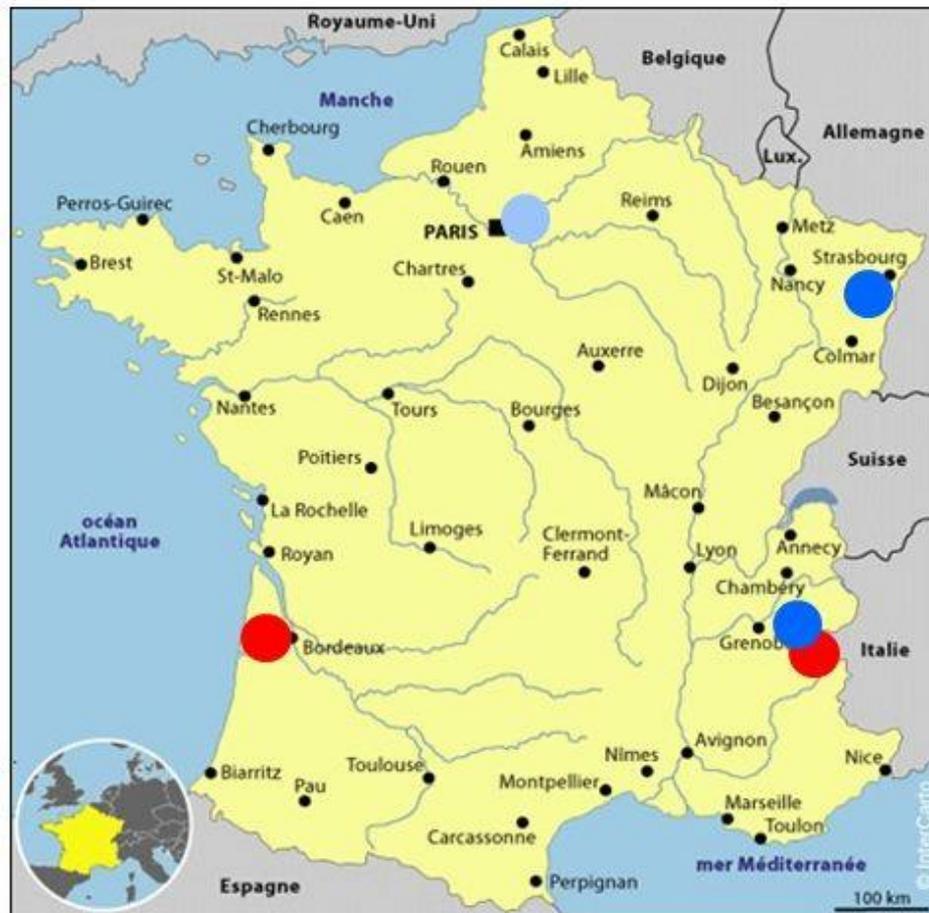
# Electron microscopy

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

2020

PIA3 France-Cryo-EM

> 2022



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

# projet EquipEx<sup>+</sup> France-Cryo-EM

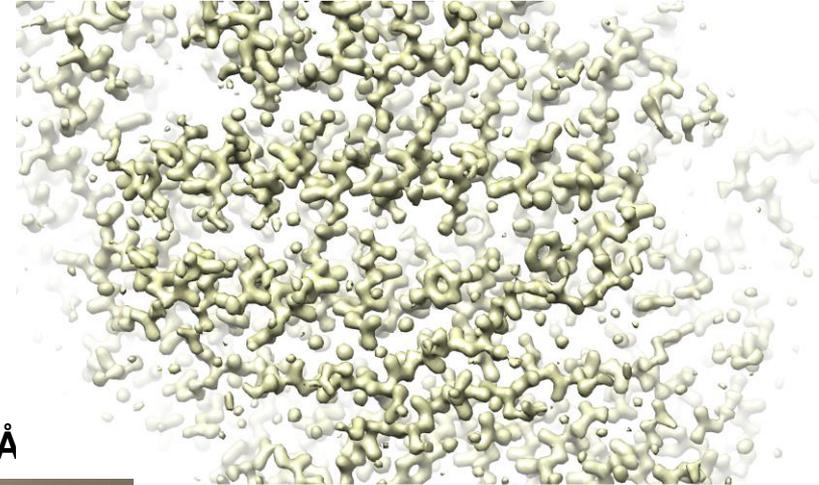
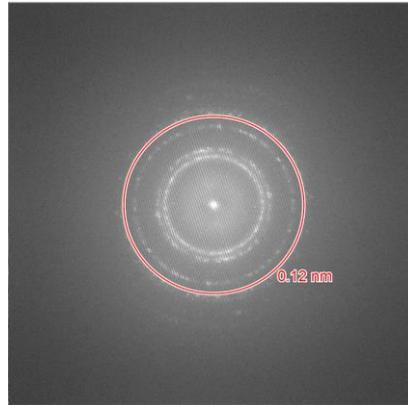
Installation of 3 Titan Krios G4 instruments at Strasbourg, Grenoble & Paris-Saclay

@CN-cryo, CBI, Illkirch/Strasbourg:

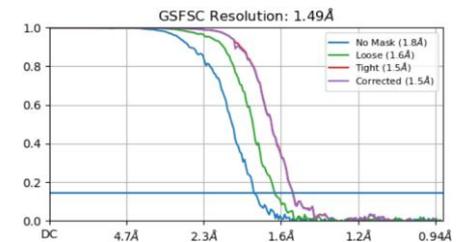
Delivered Sept. 2022, installed in dedicated CBI rooms, VA & VSR phases validated

Titan Krios 2 @CBI Illkirch/Strasbourg

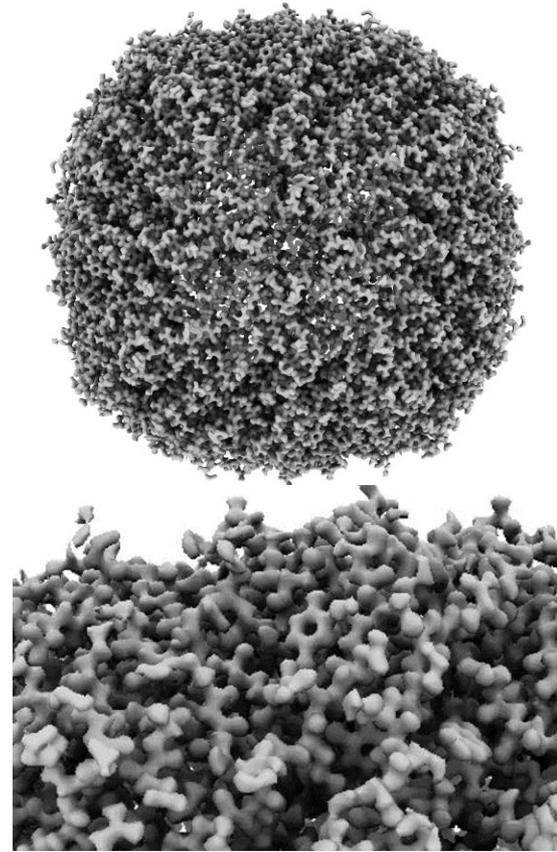
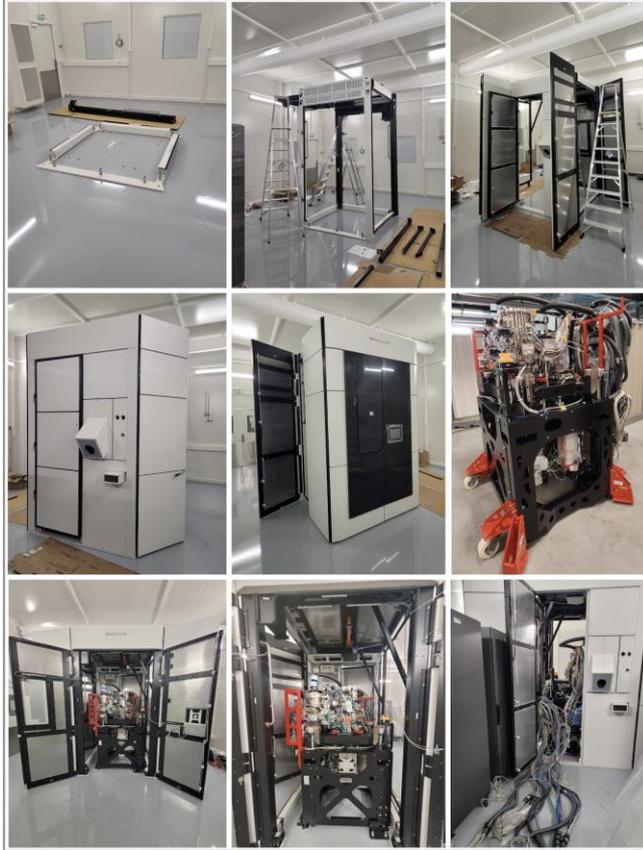
Open to the community since April 2023



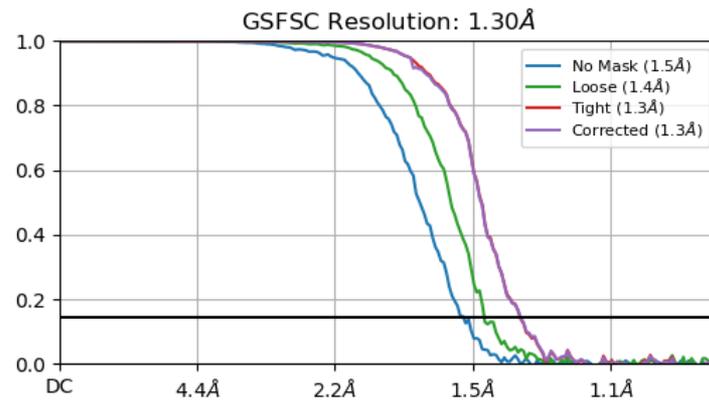
Test de resolution → >1 Å



# France cryo-EM : Krios N°2 - Grenoble



- Commissioning
- Training until end of March
- Open to user : April 2024



## Cryo-EM deployment 2024 – based on roadmap established in 2016 & initial France-Cryo-EM project

Site	Instrumentation	State
IGBMC, Ilkirch	Titan Krios 300 kV x2, Glacios 200 kV, Chameleon	Running
	Freezing device / plasma cryo-FIB	Tender / Funding pending
IBS/ESRF, Grenoble	Glacios 200 kV / Titan Krios 300 kV	Running / Final phase install
	High pressure freezer/Glacios Camera upgrade / cryo-FIB	Funded / Funded /Funding pending
SOLEIL, St-Aubin	Titan Krios 300 kV	Install Spring 2024
Pasteur Institute, Paris	Titan Krios 300 kV, Glacios 200 kV x2, cryo-FIB	Running
	BSL2 plasma cryo-FIB	Operational by 2028
	BLS3 cryo-TEM & cryo-CLEM plasma FIB	
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	Install Spring 2024
CBI, Toulouse	Talos Artica 200 kV / cryo-FIB-SEM	Running / Funding pending
IECB, Bordeaux	Talos Artica 200 kV / Glacios 2 200 kV	Running / Install Jan 2024
IGDR, Rennes	CRYO ARM 200 kV	Commissioning June 2024
IMoPA, Nancy	Glacios 2 200 kV	Install Nov 2023
ENS, Lyon	cryo-EM 200 kV	Tender pending co-funding
AFMB, Marseille	Glacios 2 200 kV	Install Jan 2024
CBS, Montpellier	High pressure freezer / cryo-FIB-SEM	Tender(s) 2023 & 2024







Cryo-EM deployment - Current status

Site	Instrumentation	State
IGBMC, Ilkirch	Titan Krios 300 kV x2, Glacios 200 kV, Chameleon	Running
	Freezing device / plasma cryo-FIB	Tender / Funding pending
IBS/ESRF, Grenoble	Glacios 200 kV / Titan Krios 300 kV	Running / Final phase install
	Glacios Camera upgrade / cryo-FIB	Funding pending
SOLEIL, St-Aubin	Titan Krios 300 kV	Install Spring 2024
Pasteur Institute, Paris	Titan Krios 300 kV, Glacios 200 kV x2, cryo-FIB	Running
	BSL2 plasma cryo-FIB	Operationnal by 2028
	BLS3 cryo-TEM & cryo-CLEM plasma FIB	
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	Install Spring 2024
CBI, Toulouse	Talos Artica 200 kV / cryo-FIB-SEM	Running / Funding pending
IECB, Bordeaux	Talos Artica 200 kV / Glacios 2 200 kV	Running / Install Jan 2004
IGDR, Rennes	CRYO ARM 200 kV	Commissioning June 2024
IMoPA, Nancy	Glacios 2 200 kV	Install Nov 2023
ENS, Lyon	cryo-EM 200 kV	Tender pending co-funding
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CBS, Montpellier	High pressure freezer / cryo-FIB-SEM	Tender(s) 2023 & 2024