



# https://frisbi.eu/

**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



MARSEILLE

STRASBOURG

PARIS-SACLAY

GRENOBLE

### 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 (co-coordinatrices C. Mas and J. Ménétrey FR SB Dark blue: Leader platforms PailsSaclay Montpellier Strasbourg Grenoble Marseille Light blue: Technology available **Spectroscopy facility** Undated Nov24 **Structure & Oligomers IBS, Grenoble** PARIS-SACLAY **Caroline MAS** Antoine ROYANT **STRASBOURG** GRENOBLE **CBS**, Montpellier **Protein Interactions & mobility IGBMC**, Strasbourg Frederic Allemand MONTPELLIER Catherine BIRCK Natacha ROCHEL MARSEILLE **AFMB**, Marseille Spectroscopies Anaïs GAUBERT EPR Maria MATÉ-PÉREZ EPR high field FTIR **I2BC, Paris-Saclay** RAMAN Stéphanie MARSIN **Binders** Super resolution microscopy FRSE Electronic Spectroscopy Agathe URVOAS in crystallo optical spectroscopy icOS Lab AnnaMaria QUARANTA Binders Spectroscopy facility **Binders** Nanobodies Alpha-Rep

# WG – Biophysical & Spectroscopy : What happened in 2024

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Binders						
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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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Nanobodies						
Alpha-Rep						

### **Equipment acquisition**

#### New EQUIPMENTS

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#### Updated EQUIPMENTS

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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

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Super resolution microscopy						
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SAXS in house					Data	
Binders						
Nanobodies						
Alpha-Rep						

## **Equipment acquisition**

#### New EQUIPMENTS

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

#### 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)



FRSB

# WG - Biophysical and Spectroscopy : Scientific Highlights



 Output

 State

#### https://www.pepr-luma.fr



AGENCE INATIONALE DE LA RECIERCIE

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

### FRISBI – Working Group

WG - Biophysical & Spectroscopy

WG - Sample Preparation

**Coordinators : A. Poterszman and R. Vincentelli** 

WG - Nuclear Magnetic Resonance

WG - Electron Microscopy

WG - Crystallization & X-ray Crystallography



https://frisbi.eu/

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

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Transient expression (hek, cho)	Mammalian						
Vaccinia virus expression	Transient expression (hek, cho)						
Large scale cultures	Vaccinia virus expression						
	Large scale cultures						
Cell Free and isotopic labelling	Cell Free and isotopic labelling						
Cell free production	Cell free production						
Wheat germ cell free expression	Wheat germ cell free expression						
Labelling	Labelling						
Library based methods	Library based methods						
Construct screening	Construct screening						
Selection of binders (Nanobody )	Selection of binders (Nanobody )						
Selection of binders (Alpha-Rep)	Selection of binders (Alpha-Rep)						
Protein purification	Protein purification						
Medium scale purification	Medium scale purification						
Small scale paralle/HTP purification	Small scale paralle/HTP purification						
Preparative Automated purification	Preparative Automated purification						

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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)



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

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Multigene construct design			
Bacterial expression	 _		
High Throughput			
Medium scale cultures (up to 6L)			
Large scale cultures (up to 70 L)			
Mammalian			
Transient expression (hek, cho)			
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Large scale cultures			
Cell Free and isotopic labelling			
Cell free production			
Wheat germ cell free expression			
Library based methods			
Construct screening			
Selection of binders (Nanobody )			
Selection of binders (Alpha-Rep)			
Protein purification			
Medium scale purification			

Genotyping			
Development stable cell lines			
Developement of knock-In cell lines *			

- HTP bacterial protein production and purification pipelines in the  $\mu g/mg$ 

scale (Marseille)



HTP Protein-protein or protein-ligand interaction detection and quantification

#### (Marseille) <sup>80</sup> human PDZ domains <sup>HTIV TAXI <sup>HTIV TAXI</sup> <sup>ODD</sup> <sup>DD</sup> <sup>D</sup></sup>



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

Physical access (Training, guidance and assistance to users) Remote access (Experiments by platform expert and sample send to users) PARIS-SAGLAY STRASBOURG GRENOBLE MONTPELLIER

Ge Nt	💑 🔹 👘 🔹 Baculovirus single and multigene
Sample Preparation	
Cloning	expression technologies (all centers)
Cloning and subcloning service	
Multigene construct design	
Bacterial expression	
High Throughput	
Medium scale cultures (up to 6L)	Bac2Bac, B2E and homologous recombination
arge scale cultures (up to /U L)	Baczbac, bzr and homologous recombination
Insect cells expression	Nultigana expression systems (NultiDae and DigDae
Baculovirus	initiagene expression systems (initiade and BigBac
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Large scale cultures	
Cell Free and isotopic labelling	(b)GBac MultiBac EastBac)
Wheat germ cell free expression	
Labelling	
Library based methods	
Construct screening	Helper
Selection of binders (Nanobody )	Kang Mini attTn7 Blue/white screening
Selection of binders (Alpha-Rep)	Bacterial growth
Small scale paralle/HTP purification	
	Or Rescham
Genotyping	Gent/Kan/Chlo/Spec/Tet Bacmid prep
Development stable cell lines	IPTG/Blu-oGal
Developement of knock-In cell lines *	St9, St21 (S.Frugiperda)
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Physical access (Training, guidance and assistance to users) Remote access (Experiments by platform expert and sample send to users)

	• Transient expression in mammalian cells (all centers)	nuc.
Cloning and subcloning service		
Multigene construct design	Transfection in evenencies of Menunchies calls (Exception	
Bacterial expression	I ransfection in suspension of Mammalian cells (Freestyle,	
High Throughput	Euri202) with algorida both arian areas called a reasonable	PARIS-SAGLAY
Medium scale cultures (up to 6L)	Expl293) with plasmids harboring mammalian promoters	
Large scale cultures (up to 70 L)	(a, a, acDNA2) with $(NAV)$ at right $(acDNA2)$	STIDA SPALLP
nsect cells expression	(e.g. pcDNAS with CMV), pTHex/pOPIN	OIIVAGEUUK
Baculovirus		
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52		
Yeast		ONENOBLE
Recombinant		
Medium scale cultures		
Large scale cultures		
Mammalian	Transient expression in insect cells (Strashourg)	IONIPELLIER
Transient expression (hek, cho)		
Vaccinia virus expression		
Large scale cultures	Transient transfection in insect cells (Hi5)	MARSEILLE
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Cell free production	with plasmids harboring insect promoters	
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Labelling		
Construct screening		
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Protein purification		
Medium scale purification		
Small scale paralle/HTP purification		
Preparative Automated purification		
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Large scale cultures		The state of the second s

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

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Cell Free and isotopic labelling							
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Protein purification							
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Development stable cell lines							
Developement of Knock-In cell lines *							

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

- 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)





Tubulin α,β

Chevrel A et al. J. Struct. Biol. (2018) Campanacci V et al. Structure (2019) PNAS (2019); PNAS (2022)

Dark blue: National entry point per technology						
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Cloning and subcloning service						
Multigene construct design						
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Insect cells expression						
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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)

- 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 PARIS-SAGLAY STRASBOURG GRENOBLE MONTIPELLIER MARSEILLE



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



### FRISBI – Working Group

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WG - Sample Preparation

WG - Nuclear Magnetic Resonance

**Coordinator : N. Sibille** 

WG - Electron Microscopy

PARIS-SACLAY STRASBOURG GRENOBLE MONTPELLIER MARSEILLE

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WG - Crystallization & X-ray Crystallography

# 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)
- <sup>31</sup>P and <sup>19</sup>F optimised solution probes
  - ✓ 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



HR MAS probes



FRS

PARIS-SACLAY

MONTPELLIER

GRENOBLE

**STRASBOURG** 



# WG – NMR : Resource Catalogue

In-cell NMR



Refrigerated sample changer for metabolomics





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

#### **High-pressure NMR**







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

## Daedalus system : ZrO<sub>2</sub> NMR tube + Pump (up to 3000 bar)

Available for all NMR spectrometers in ICSN site

1229

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.





### FRISBI – Working Group

WG - Biophysical & Spectroscopy

WG - Sample Preparation

WG - Nuclear Magnetic Resonance



WG - Electron Microscopy

Coordinators : B. Klaholz and G. Schoen

https://frisbi.eu/

WG - Crystallization & X-ray Crystallography



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



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



Contact : <u>cm02.contact@ibs.fr</u>

Pauline Juyoux dans l'audience



FRISI France-Cryo-EM : Krios N°2 - Grenoble Krios CM02 (installed @ESRF managed by IBS/ISBG) **Open to the community January 2025** PARIS-SACLAY Cryo-FIB : Aquilos 2 **STRASBOURG** GRENOBLE MONTPELLIER MARSEILLE Lift out iFLM Ouverture aux utilisateurs 2<sup>ème</sup> – 3<sup>ème</sup> Trimestre 2025 EM Glacios 1 / ICE instruct Falcon 4i / K2 Disponible

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

Installation of 3 Titan Krios G4 instruments France-Cryo-EM : Krios N°3 - SOLEIL at Strasbourg, Grenoble & Paris-Saclay Krios CM03 (installed @SOLEIL **Open to the community November 2024** PARIS-SACLAY Control **STRASBOURG** PX1 PX2A Users GRENOBLE **KRIOS** MONTPELLIER DISCO lab (100 m P. LEGRAND - AFC-Montpellier - 4/07/2024 Fourier Shell Correlation & docking; CWAT 12/23 MARSEILLE GSFSC Resolution: 1.11Å 1.0 No Mask (1.3Å) Loose (1.2Å) 0.8 Tight (1.1Å) Corrected (1.1Å) instruct 0.6 0.4 0.2 0.0 DC 4.3Å 2.2Å 1.4Å 1.1Å

ARIA

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

#### Entrée commune ana GET STARTED projets SUNset (envisagé) 13000 3 0 0 0 Besearch infrastructures and Européens EC Funded Projects SOLEIL Users Net ARIA is a cloud platform for Access and Facility management "Office 365 for Research Infrastructures", ARIA provides a Jame suite of tools t manage your RI and offer your services to resear Weigene to the SOLETL User Net set This is your space to submit your proposals, to and publications submission and many more. L New SUN set User Registered SUN set Us ever registered at SOLEIL as a User to create a ation has to be done welv over Enter here to log in to the SUN set. t-ERIC built ARIA based on years of experience delivering access to cutting edg ties armss Furnne, ARIA has since been used by FC Funder! projects and national facilitie Lost Password and is now the go-to solution for access provision and facility manager ot remember your SUN set username and/or prompted for your email address and the SUN set information. Please contact SOLEIL user office in

Supported & optimized for Mozilla Firefox & Google Chrome browsers Please do not use the back and forward buttors of your browsers inside the SUN set.

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.





#### 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	
	Titan Krios 300 kV I & II, Glacios 200 kV, Chameleon	Running	
IGBMC, llkirch	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	
Dastour Instituto Daris	Titan Krios 300 kV, Glacios 200 kV x2, cryo-FIB	Running	
Pasteur Institute, Paris	BSL2 plasma cryo-FIB, BLS3 cryo-TEM & cryo-CLEM plasma FIB	Operationnal by 2028	
Curio Instituto Doris	Glacios 2 200 kV / High speed high pressure freezer	Running	
Curie Institute, Paris	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	

FRSB PARIS-SACLAY **STRASBOURG** GRENOBLE **MONTPELLIER** MARSEILLE

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



# WG – Crystallization & X-ray crystallography : Resource Catalogue

#### 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-crystallization					
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					

Controlled atmosphere in crystal growth and Cubic-phase crystallization (Grenoble)

**PARIS-SACLAY** 

MONTPELLIER

GRENOBLE

**STRASBOURG** 

MARSEILLE

- 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

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

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Automatic pipetting					
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Nanoliter pipetting					
Manual refinement	advices to users				
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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-crystallization					
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					

# FRIS **Equipment** acquisition **New EQUIPMENTS** RockImager 360UV (Strasbourg) PARIS-SACLAY **STRASBOURG** GRENOBLE MONTPELLIER MARSEILLE

#### Training

#### 2024

- FRISBI members continue to be very active in RÉCIPROCS-bio
- RÉCIPROCS-bio, Micro-ED school, Montpellier, June 2024
- RÉCIPROCS-bio, TEA-BAG, User meeting Soleil 2024
- RÉCIPROCS-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					
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-crystallization					
Nanobodies	users bring their				
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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					

### **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

# PARIS-SAGLAY GRENOBLE MONTPELLIER MARSEILLE

# Training

#### 2024

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

#### In 2025 (September)

 ANF (Action nationale de Formation) in preparation for 2025, SOLEIL - by RÉCIPROCS-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.

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