New Opportunities for Integrated Structural Biology at 4th generation synchrotron sources.

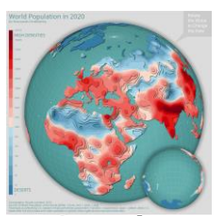
Andrew Thompson, Renafobis webinar 1st March 2022.

Outline.

1. What do I understand by Integrated Structural Biology.
2. Integrative Structural Biology at SR sources – examples combining methods.
3. Taking advantage of ultra-brilliant radiation source : HELIOBIO vision for integrated structural biology in the context of the SOLEIL Upgrade.
4. New methods at SOLEIL and enabling technologies – microfluidics, cryo-sample environment, machine learning/data mining.
5. Conclusions.

“Exploring ‘life in context’”

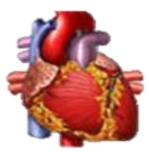
“Molecular biologists […] **study the world at the smallest of scales**: chromosomes, subcellular structures, proteins, metabolites. But, too often, **this focus and our well-controlled labs deprive us of the fullest picture**.”

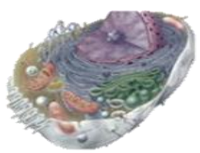

Species global


Species local

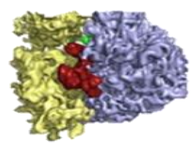

Population

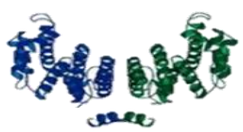

Organisms


Tissue


Cells


Organelles


Complexes


Proteins

Atoms

“Molecular biologists are used to **multidisciplinarity**: we deploy **X-ray physics** and **cryo-electron microscopy** to study **DNA, RNA and protein structures**; chemistry to understand metabolic pathways; and informatics to analyse variation, including genomes and their epigenetic modifications. We can measure **metabolites at the single-cell level**, and we use fluorescence to identify **cell organelles and macro-molecules in multicellular systems**. These data integrate genetic variation with phenotypic variation in individual cells, revealing associations that show how microbes (and other cells) function in different conditions.”

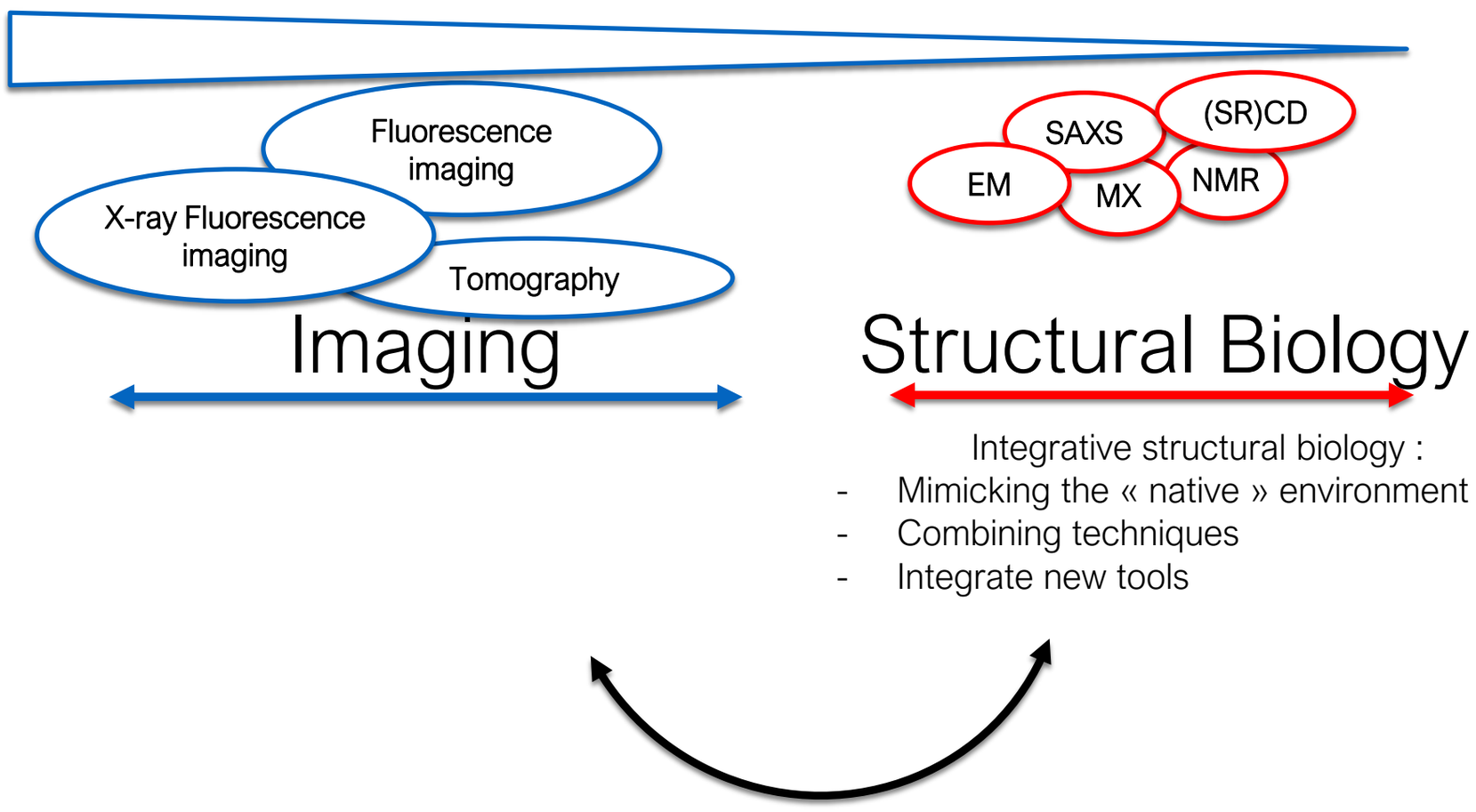
Nature, January 2022

World view

Molecular biologists: let’s reconnect with nature

By Edith Heard

Connecting the molecular and the cellular levels.



How to connect both worlds to cross the scales?

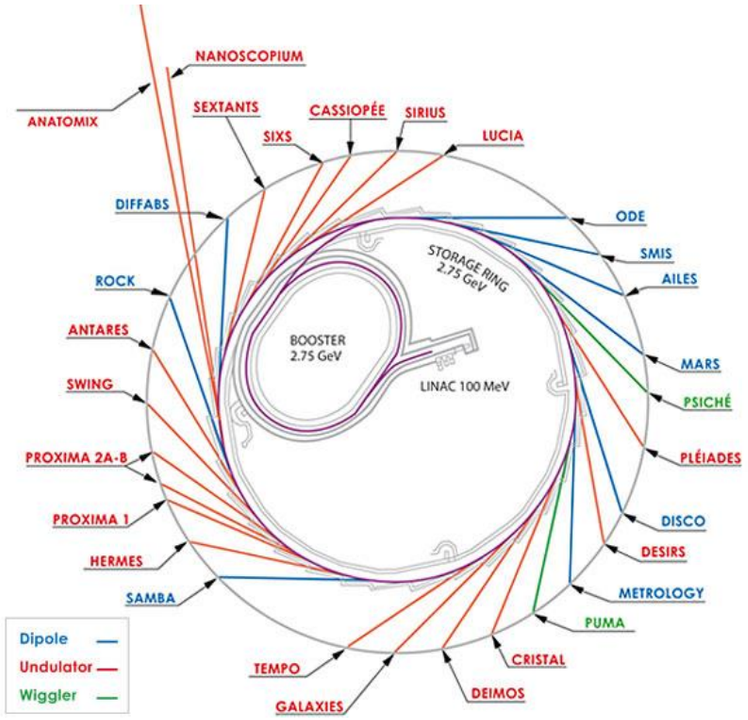
What do I understand by ‘integrative structural biology’?

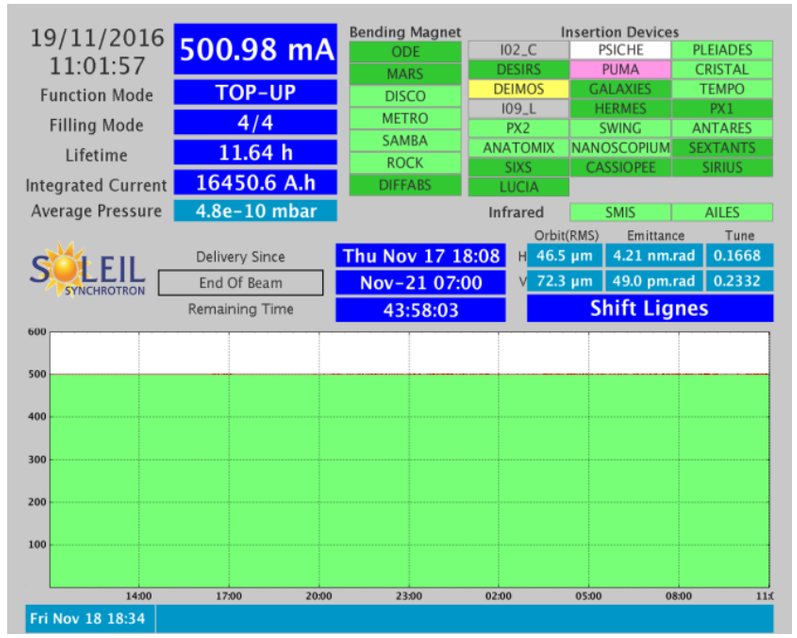
Extract from SOLEIL conceptual design report science case :

- ***As structuralists, we want to understand how life works from atomic detail right through to individuals. This implies seeing life at its different levels of organisation (using non-invasive methods) in 3D, in vivo/under physiological conditions, in real time, at atomic resolution and with control over the biomolecular environment (mutations, control of gene expression, cell-cycle control, pathogens……)***

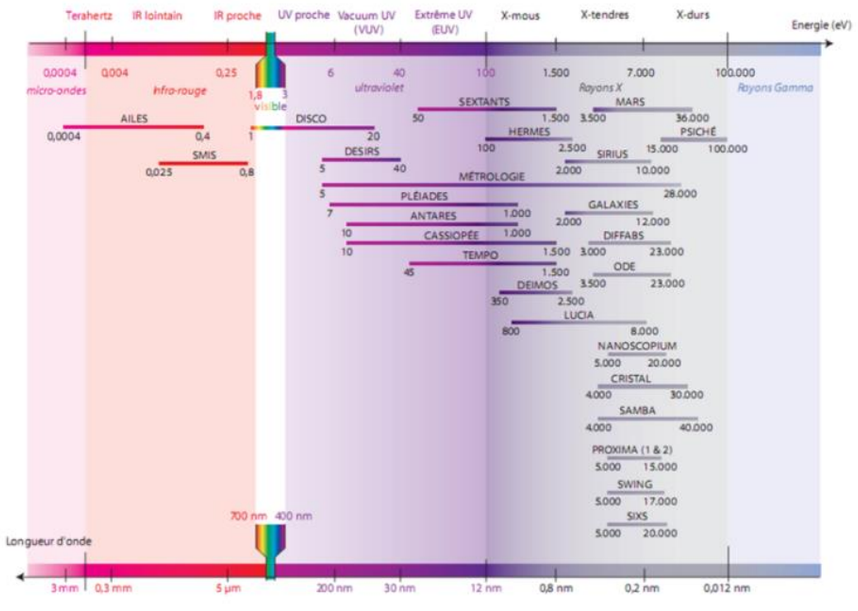
Today, this is an insoluble problem. But by combining methods, understanding the meaning and limitations of the results of each and how they apply to the system under study, we can get closer.

SOLEIL – a 3rd generation synchrotron source

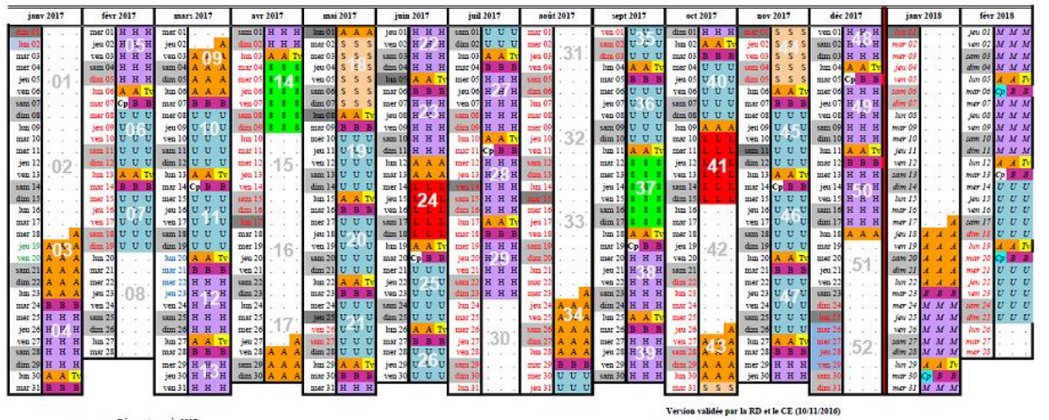




29 Beamlines open to users



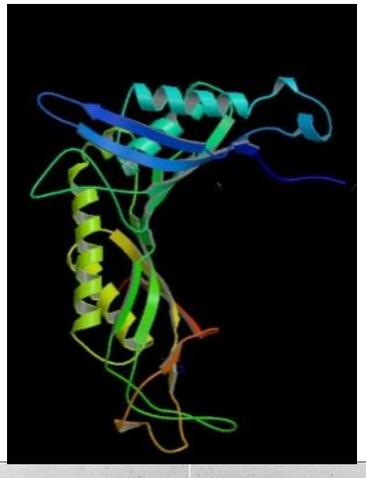
Calendrier de fonctionnement 2017

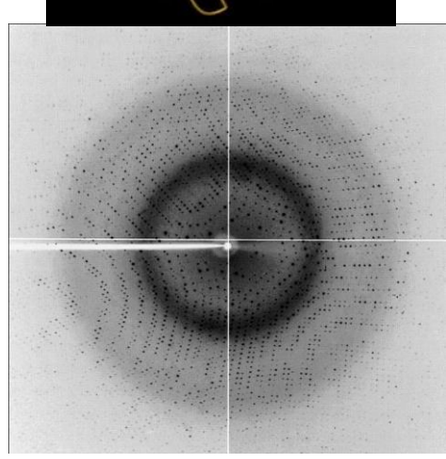


Large spectral range - different radiation interactions with matter.

Fluorescence

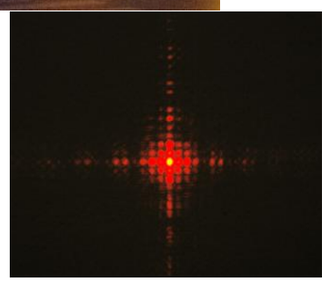
scattering





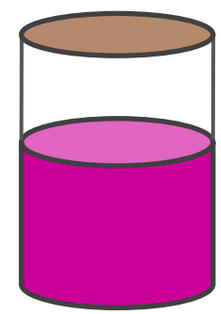
Photoemission

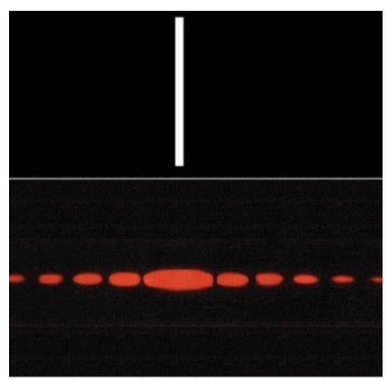
transmission



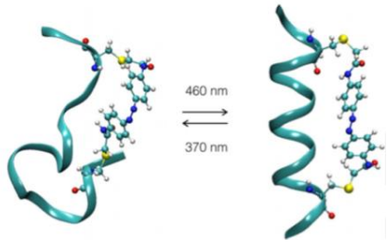
absorption

diffraction

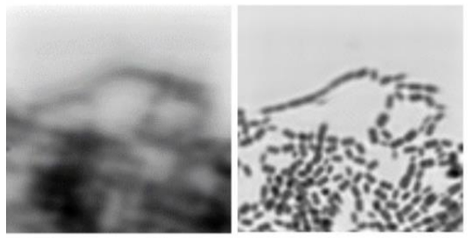




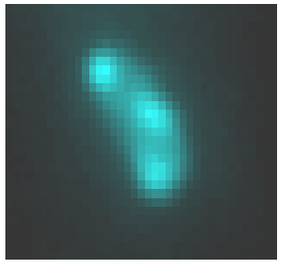
DISCO

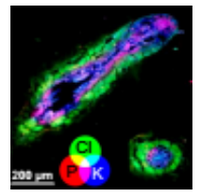


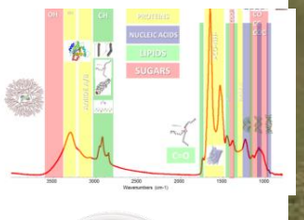
HERMES



LUCIA







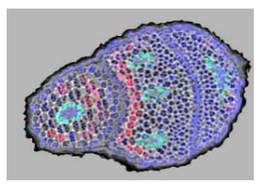
Soft
X-Ray
Imaging
Hard

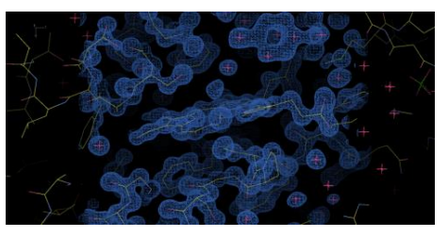
SMIS

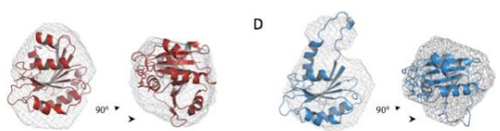
PROXIMA 1

PROXIMA 2

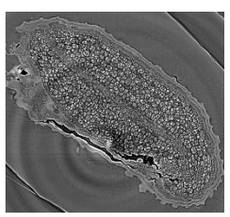
NANOSCOPIUM







SAXS - WAXS - GISAXS
SWING



anatomix

Example 1 – Antibody binding

Nature, 2015 and 2016.

Recognition determinants of broadly neutralizing human antibodies against dengue viruses

Alexander Rouvinski[1,2]*, Pablo Guardado-Calvo[1,2]*, Giovanna Barba-Spaeth[1,2]*, Stéphane Duquerroy[1,2,3], Marie-Christine Vaney[1,2], Carlos M. Kikuti[1,2]†, M. Erika Navarro Sanchez[1,2]†, Wanwisa Dejnirattisai[4], Wiyada Wongwiwat[4], Ahmed Haouz[5], Christine Girard-Blanc[5], Stéphane Petres[5], William E. Shepard[6], Philippe Desprès[7]†, Fernando Arenzana-Seisdedos[8], Philippe Dussart[9]†, Juthathip Mongkolsapaya[4,10], Gavin R. Screaton[4] & Félix A. Rey[1,2,5]

ARTICLE

doi:10.1038/nature18938

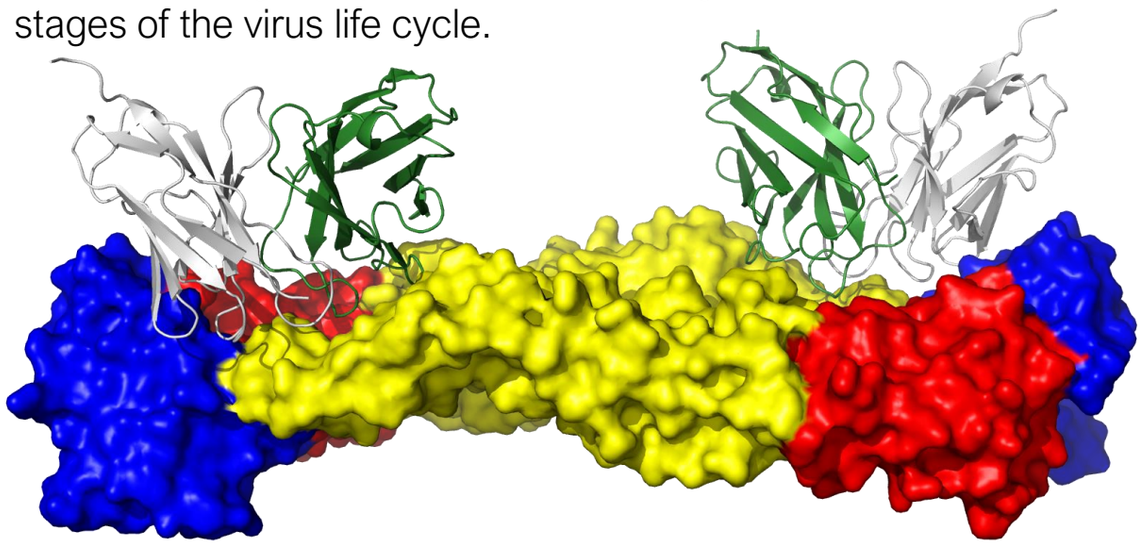
Structural basis of potent Zika–dengue virus antibody cross-neutralization

Giovanna Barba-Spaeth[1,2]*, Wanwisa Dejnirattisai[3]*, Alexander Rouvinski[1,2]*, Marie-Christine Vaney[1,2]*, Iris Medits[4], Arvind Sharma[1,2], Etienne Simon-Lorière[5,6], Anavaj Sakuntabhai[5,6], Van-Mai Cao-Lormeau[7], Ahmed Haouz[8,9], Patrick England[9,10], Karin Stiasny[4], Juthathip Mongkolsapaya[3,11], Franz X. Heinz[4], Gavin R. Screaton[3] & Félix A. Rey[1,2]

- 4 serotypes (DENV 1-4) of the virus. No vaccine for all 4 serotypes. Secondary infection likely to cause more serious symptoms (antibody dependant enhancement).

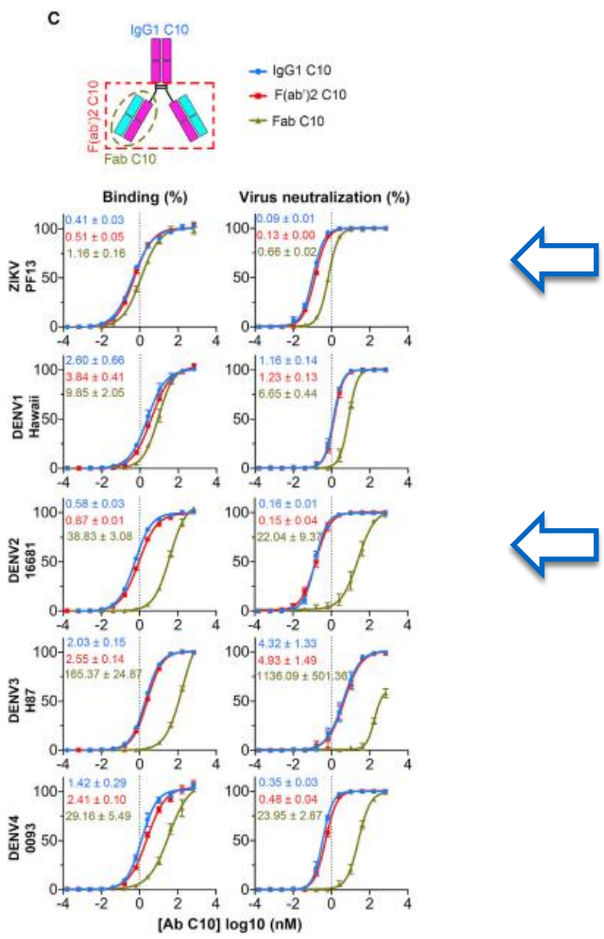
- Screaton et al (2015, Nature Immunology) produced monoclonal antibodies from blood samples of 7 patients. Some found to be broadly neutralising. Rey et al. performed a structural characterisation of 4 broadly neutralising antibodies. Further work showed that 2 were cross reactive for Zika.

- Antibodies target E protein, which changes conformation at different stages of the virus life cycle.



The epitope arrangement on flavivirus particles contributes to Mab C10's extraordinary neutralization breadth across Zika and dengue viruses

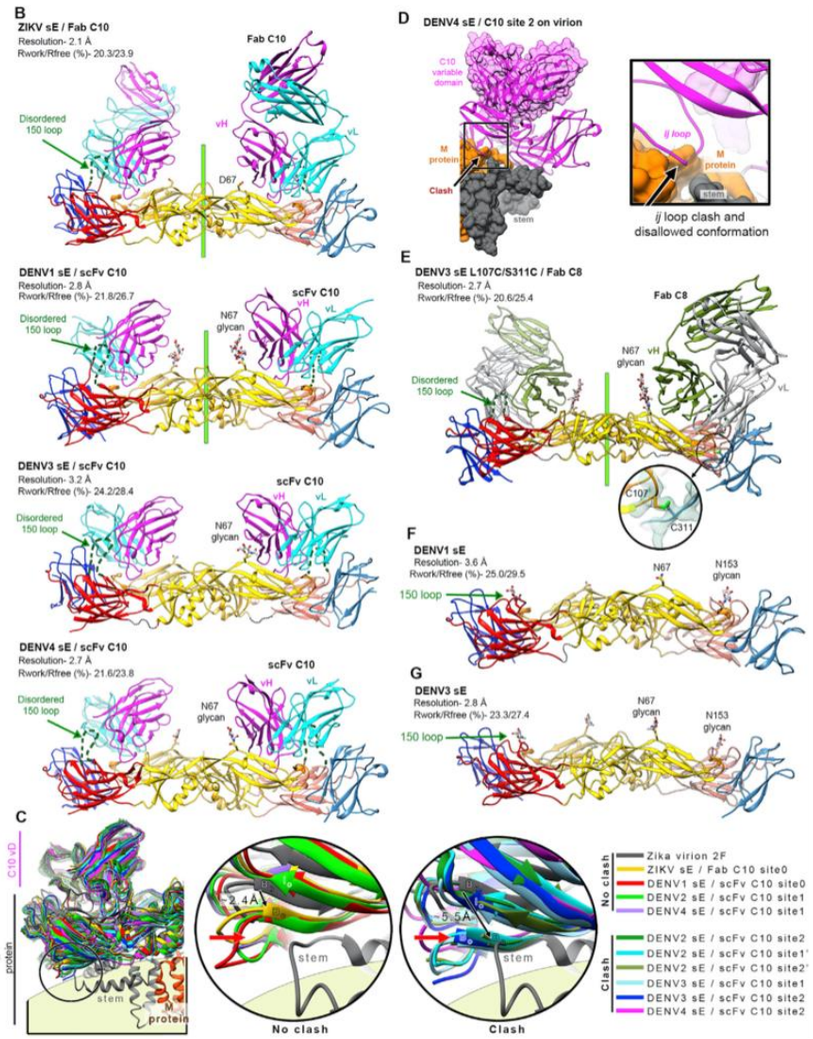
Sharma et al, Cell, December 2021.



To understand the origin of cross neutralisation over 5 different flaviviruses (4 serotypes of dengue and Zika), and neutralising capacity of bivalent vs monovalent binders, needed to compare X-ray and Cryo-EM structures (Zika – Sevvana et at 2018, Denv2 – Hardy et al 2021, complex Denv2 – C10 scFv).

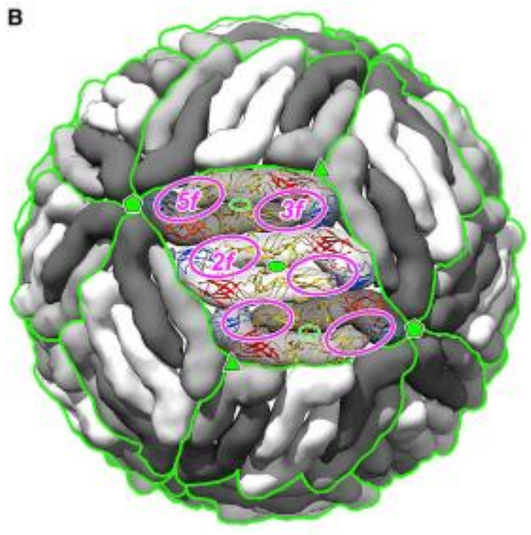
X-ray crystallography of different envelope protein/antibody complexes was performed at several SR sources (ESRF, SLS and SOLEIL).

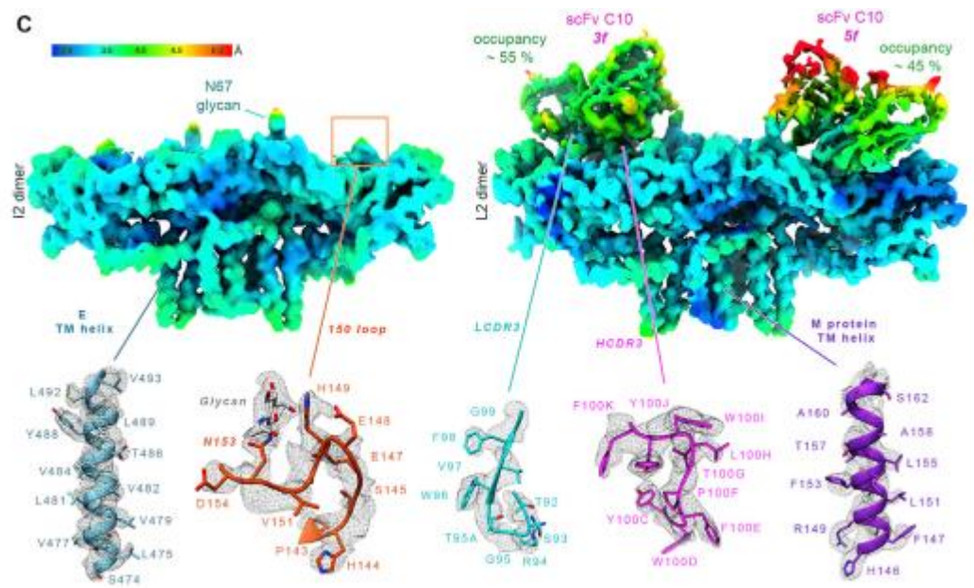
Alignment of crystal and Cryo-EM structures.



Crystal structures of sE from 5 viruses with C10 Fa and scFv. Structures show symmetrical dimers for ZikV and DENV1 (compatible with existing 3.1 A Cryo-EM structure of ZikV), but asymmetric for DENV 2- 4 . In this latter case only the binding of 1 half dimer was compatible with existing 2.6 A Cryo-EM structure of DENV2.

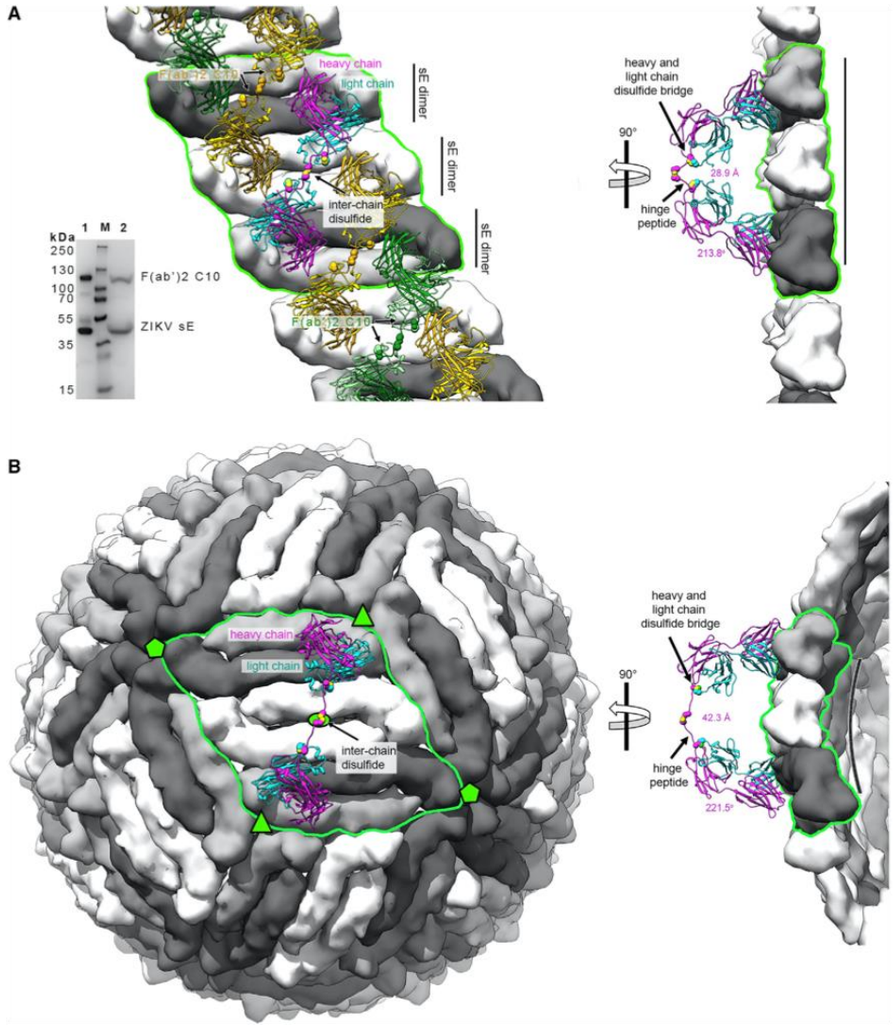
Overall virion composed of 90 E dimers making up 30 ‘rafts’. The binding epitopes are situated in 3 non equivalent environments.





Cryo-EM structure of DENV2 virion with C10 scFV (3.3 A) shows binding at only two of four epitopes, contrasting with ZIKV / FAB structure at ~4 A resolution which showed even coverage at all epitopes.

Crystallographic evidence for bivalent binding mode linking two different dimers.



Electron density for hinge region in ZIKV sE in complex with both antibody and Fab indicates bivalent binding model, compatible with and modelled on curved virus surface.
Suggests that 30 antibodies can completely coat the mature virus surface.
Geometry of hinge region taken with Cryo-EM data suggests that only 3f epitopes can bind bivalently and remain stably bound – since antibody neutralizes DENV2 and 4 as strongly as ZIKV.

The combination of crystallography and Cryo-EM SPA of antibody complexes with Denv2 and Zika viruses (one example of each neutralizing class) demonstrate the importance both of epitope organization at the surface of the virion and the interaction of the antibody with its epitope to the broad neutralization capacity of MAB C10s.
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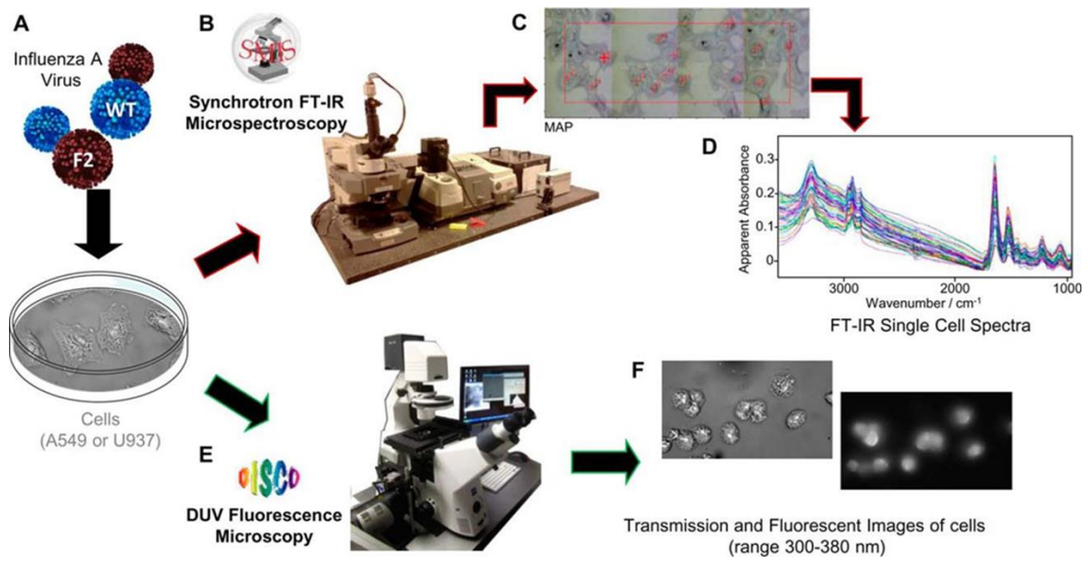
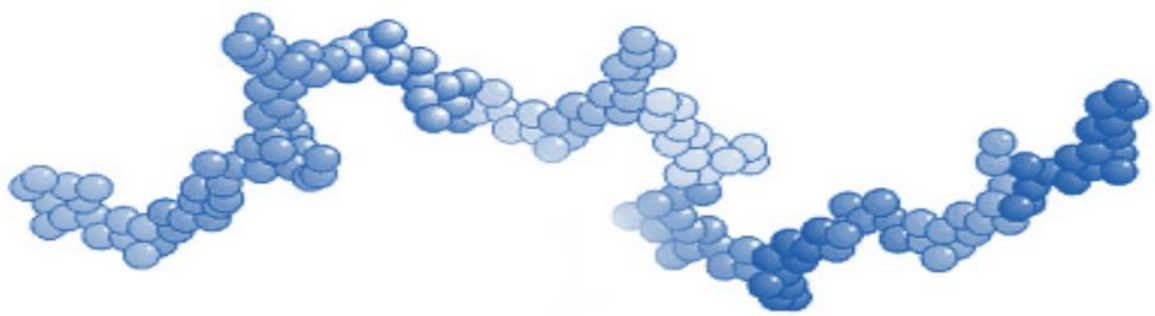
Example 2 – From molecular organization to pathogenicity

Synchrotron FTIR imaging and DUV imaging

- PB1-F2 : a virulence factor of influenza A virus, known to increase pathogenicity in mammals.
- Full length forms expressed in H5N1 avian strains and some previous H1N1 pandemic strains.
- Molecular mechanism associated with increased pathogenicity is unknown.
- Intrinsically disordered protein, unstructured in aqueous solution but can switch to alpha helical or beta sheet structure depending on hydrophobicity.
- Strong propensity to oligomerize.

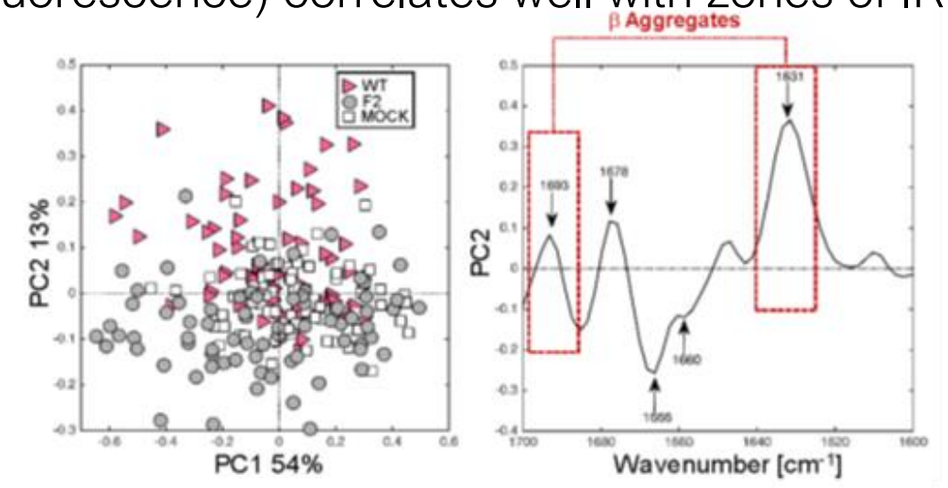


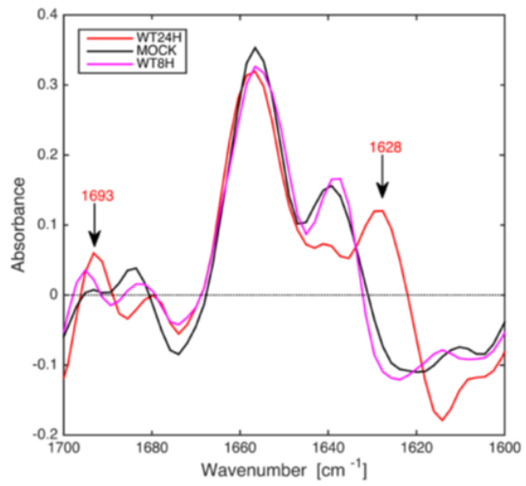


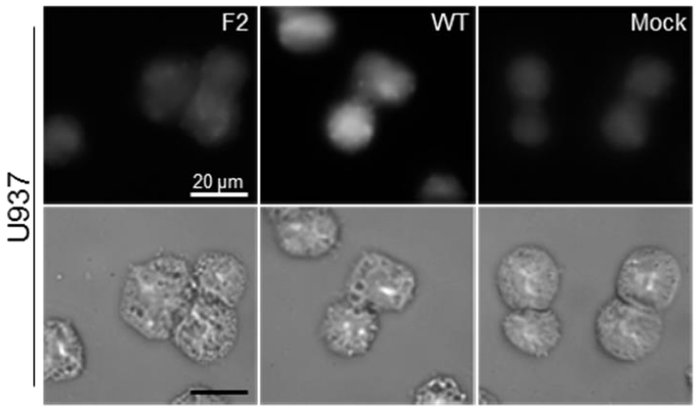
VIM Inra, Jouy en Josas based on a series of publications from Chevalier et al (2016, 2021) and LeGoffic et al (2011).

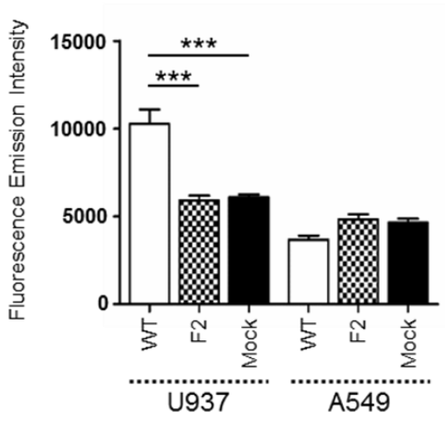
Detection of amyloid fibres of PB1-F2 in IAV infected cells

- Human pulmonary epithelial cells (A549) and monocytic cells (U937).
- Infection with wild type (WT) or PB1-F2 knock out mutant (F2).
- FTIR spectra (8h post infection) show presence of β – aggregates in U937 but not in A549.
- Take advantage of fact that PB1-F2 contains an unusually high concentration (>5%) of tryptophan residues.
- DUV microscopy images (wavelength of 280 nm chosen to highlight W autofluorescence) correlates well with zones of IR detected aggregates.









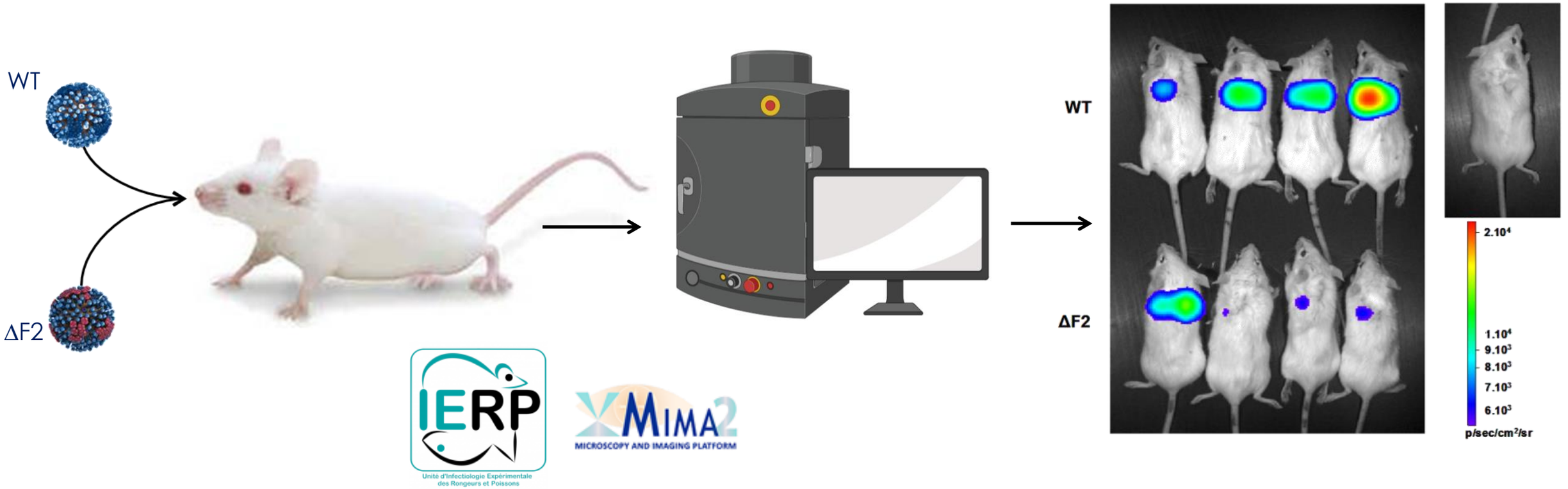
Chevalier et al. J. Biol. Chem. 2016

Detection of amyloid fibres of PB1-F2 in the lungs of IAV infected mice

NF-κB - luciferase transgenic mouse model

Mice nasally instilled with monomeric, fibrillated or truncated forms of PB1-F2

WT instilled forms show bioluminescence (after luciferin instillation) corresponding to lung inflammatory response 3 days post infection.



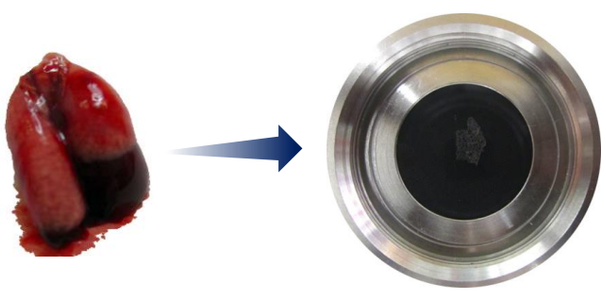
IERP Unité d'Infectiologie Expérimentale des Rongeurs et Poissons

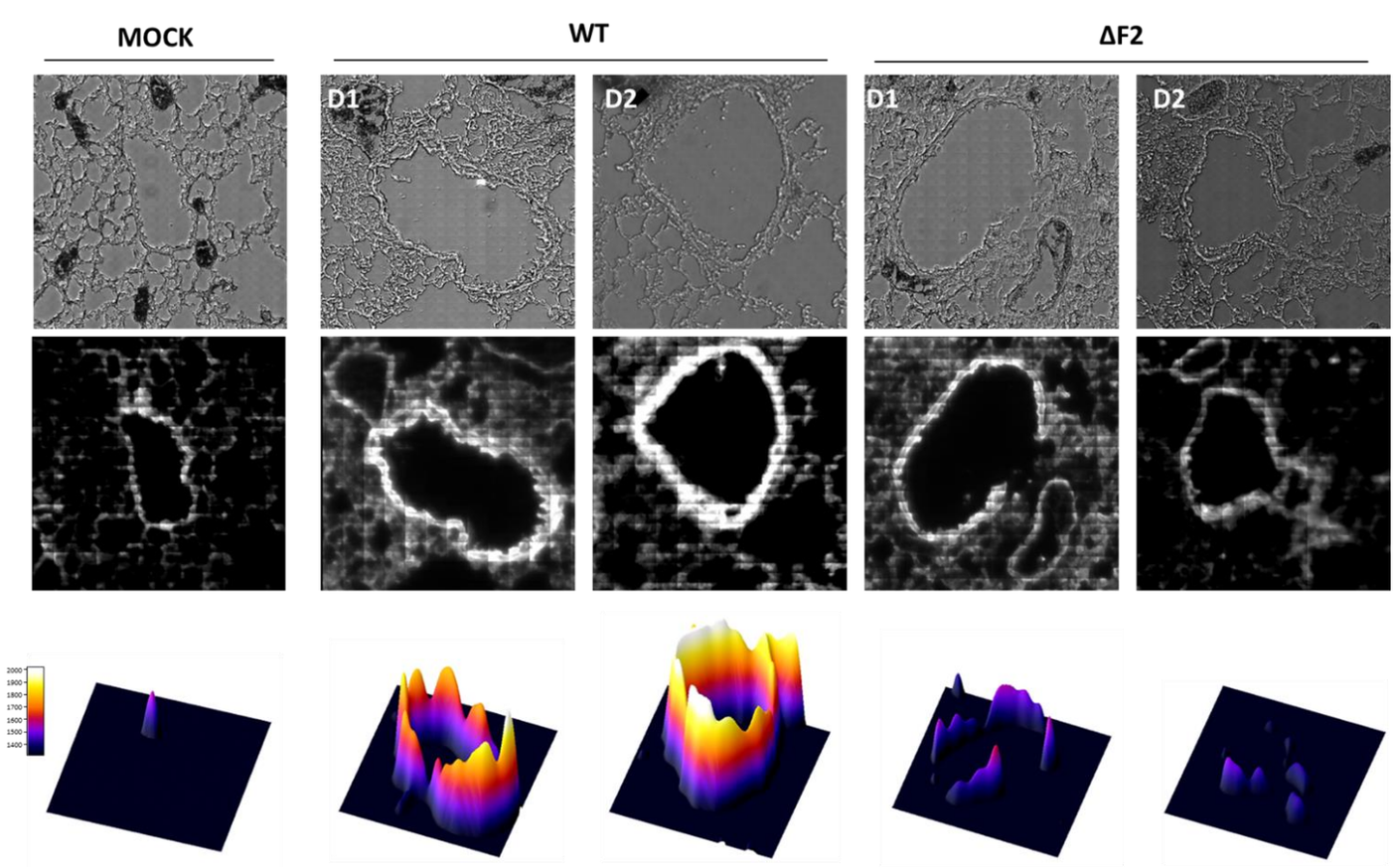
MIMA2 MICROSCOPY AND IMAGING PLATFORM

Le Goffic et al. PLoS Path. 2011

Multimodal approach - DUV

Use DUV microscopy to identify ROI in mouse lungs 1 and 2 days post infection.

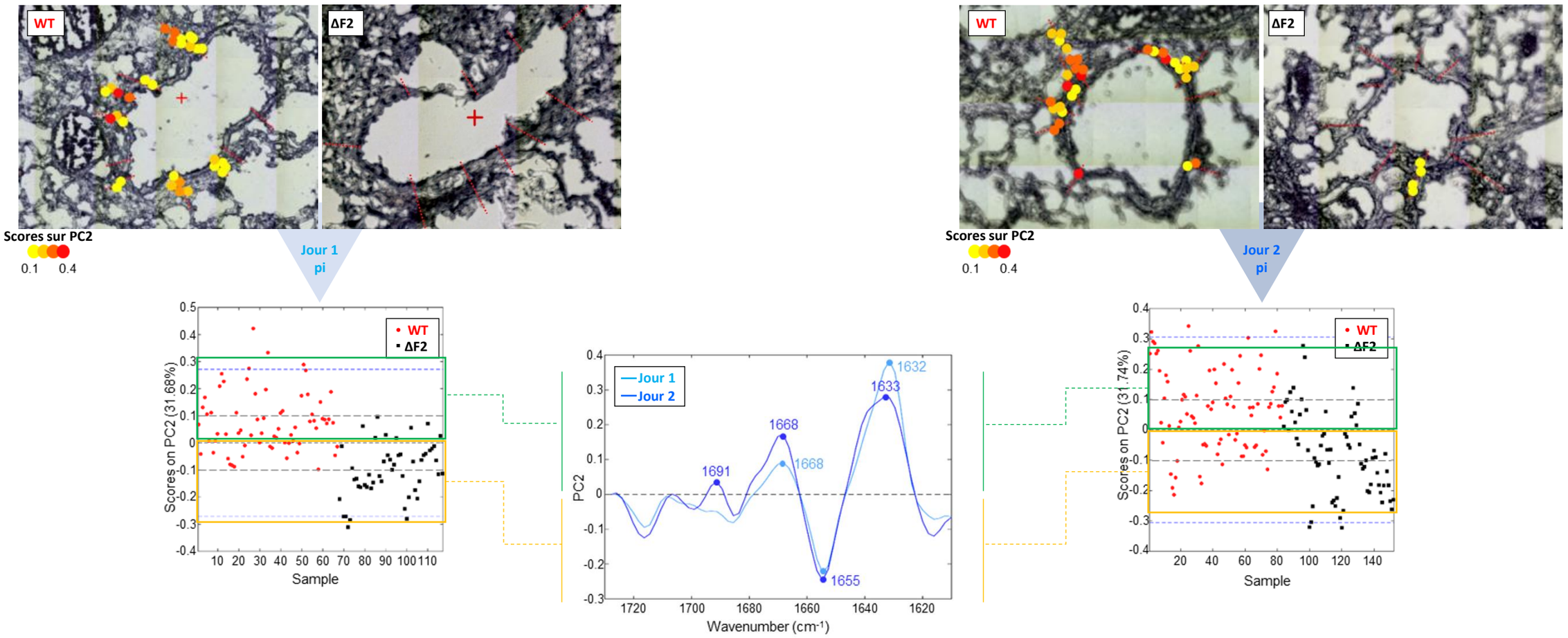




Chevalier et al. JBC. 2021

Multimodal approach - IR

Then use FTIR spectroscopy to investigate the correlation of inflammation and β-aggregate formation

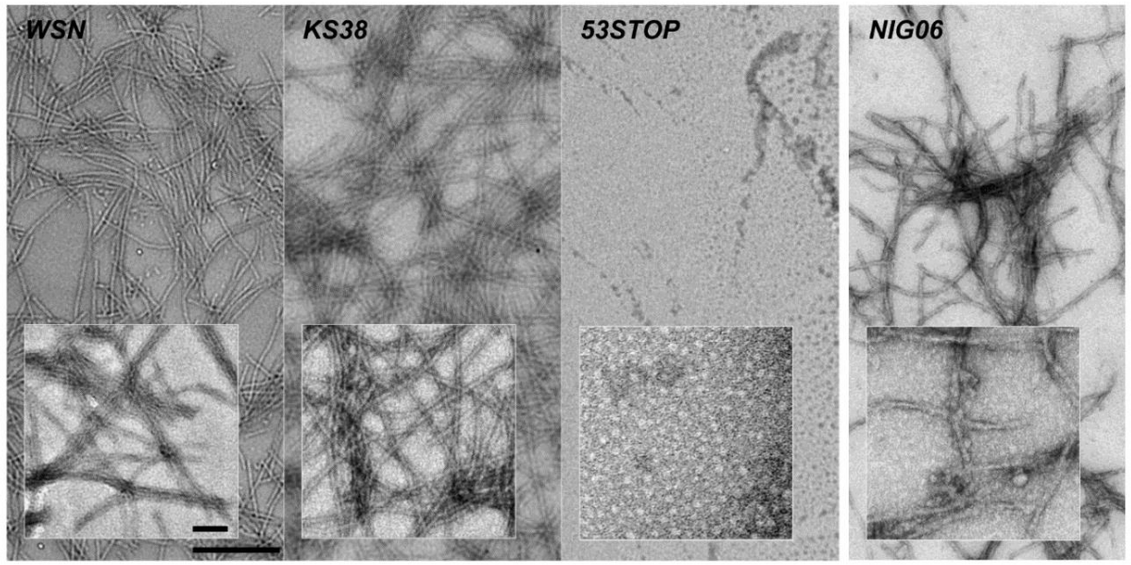


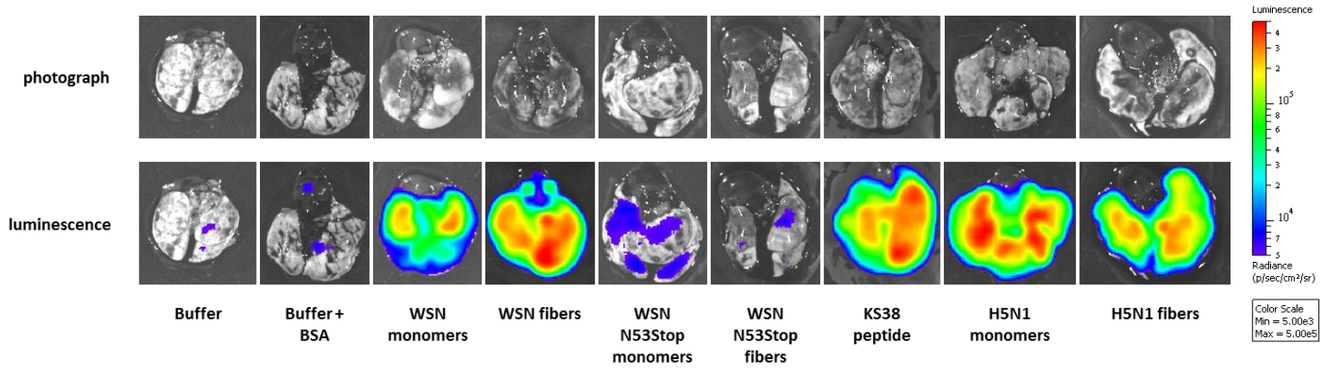
Chevalier et al. JBC. 2021

Recombinant PB1-F2 and the inflammatory reponse

Molecular characterisation correlated with pathogenicity.

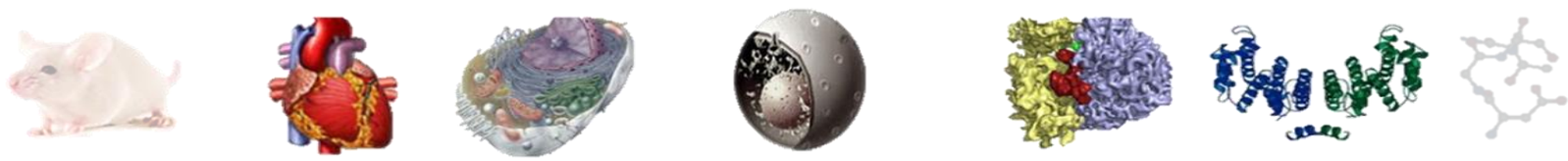
- Degree of fibrillation of lungs examined by transmission electron microscopy.
- PB1-F2 fibres are recognised by the immune system leading to inflammatory response.
- Oligomerisation of PB1-F2 also negatively impacts airways.





Chevalier et al. JBC. 2021

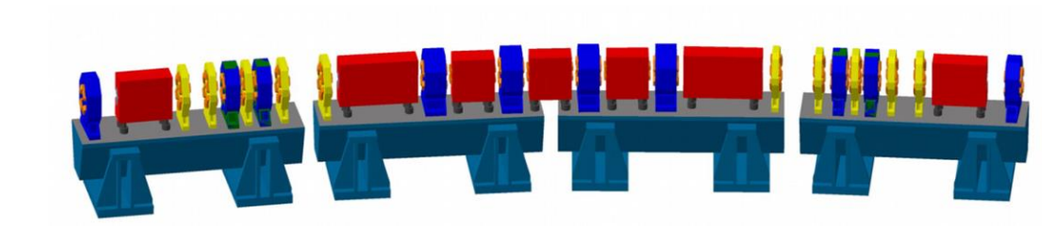
SOLEIL Upgrade



Organisms Tissue Cells Organelles Complexes Proteins Atoms

SOLEIL Upgrade and its opportunities

- 20 years of user operation in 2028, upgrade (2 x 5 year phases) of accelerators, beamlines and infrastructure.
- Address present and future scientific and societal challenges via a reconstruction of the storage ring (emittance 50 x 50 pm.rad, 100x brighter beams).
- Routine access to nanometer scale and dynamics of processes ; development of entirely new experimental methods.
- Upgrade of IT infrastructure in a data centered approach. Upgrade ageing infrastructure to reduce environmental footprint.



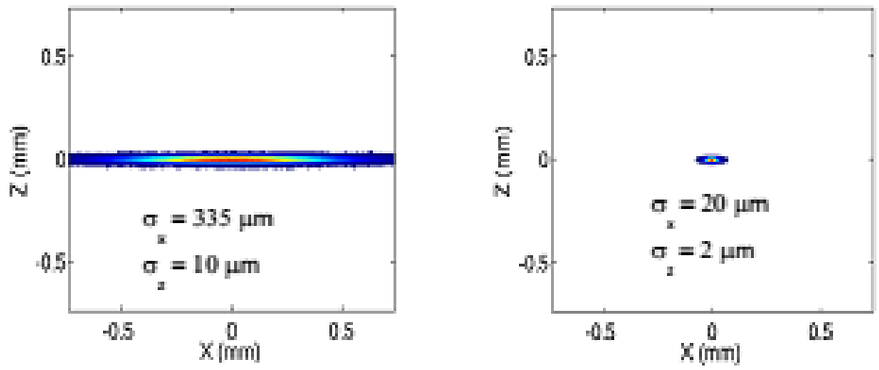
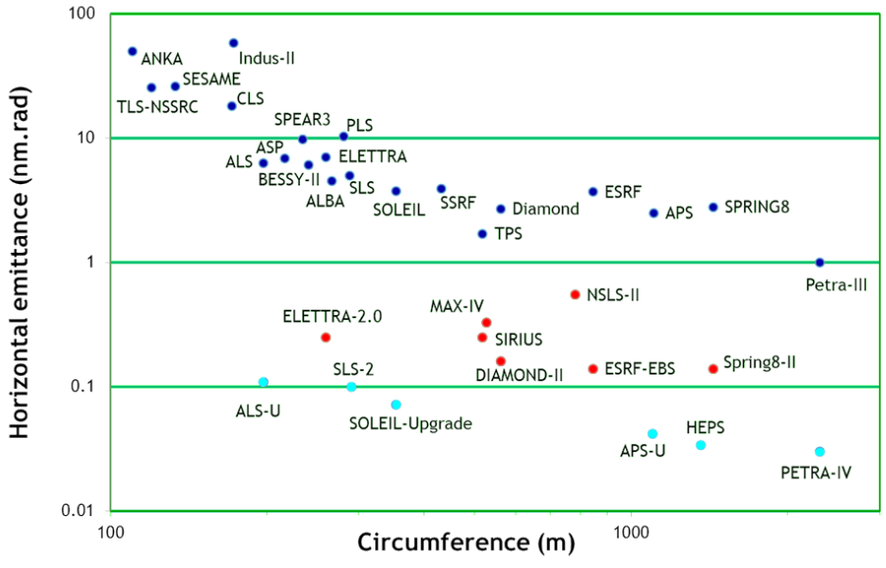
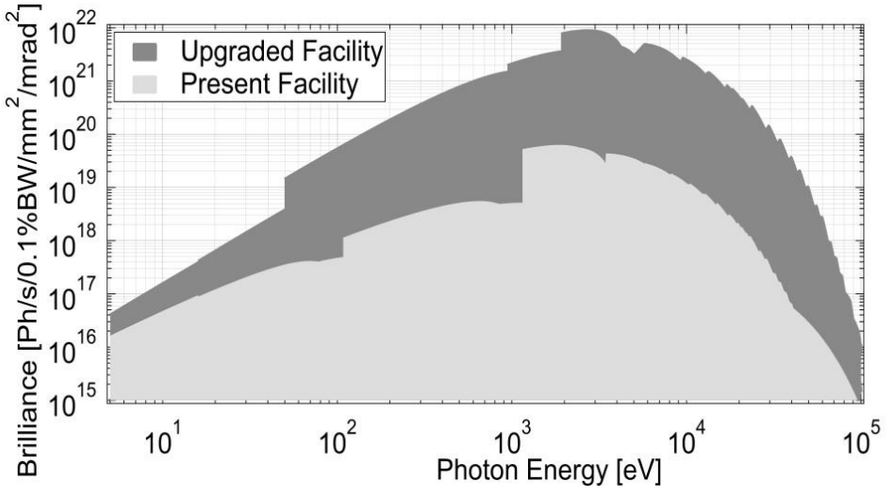
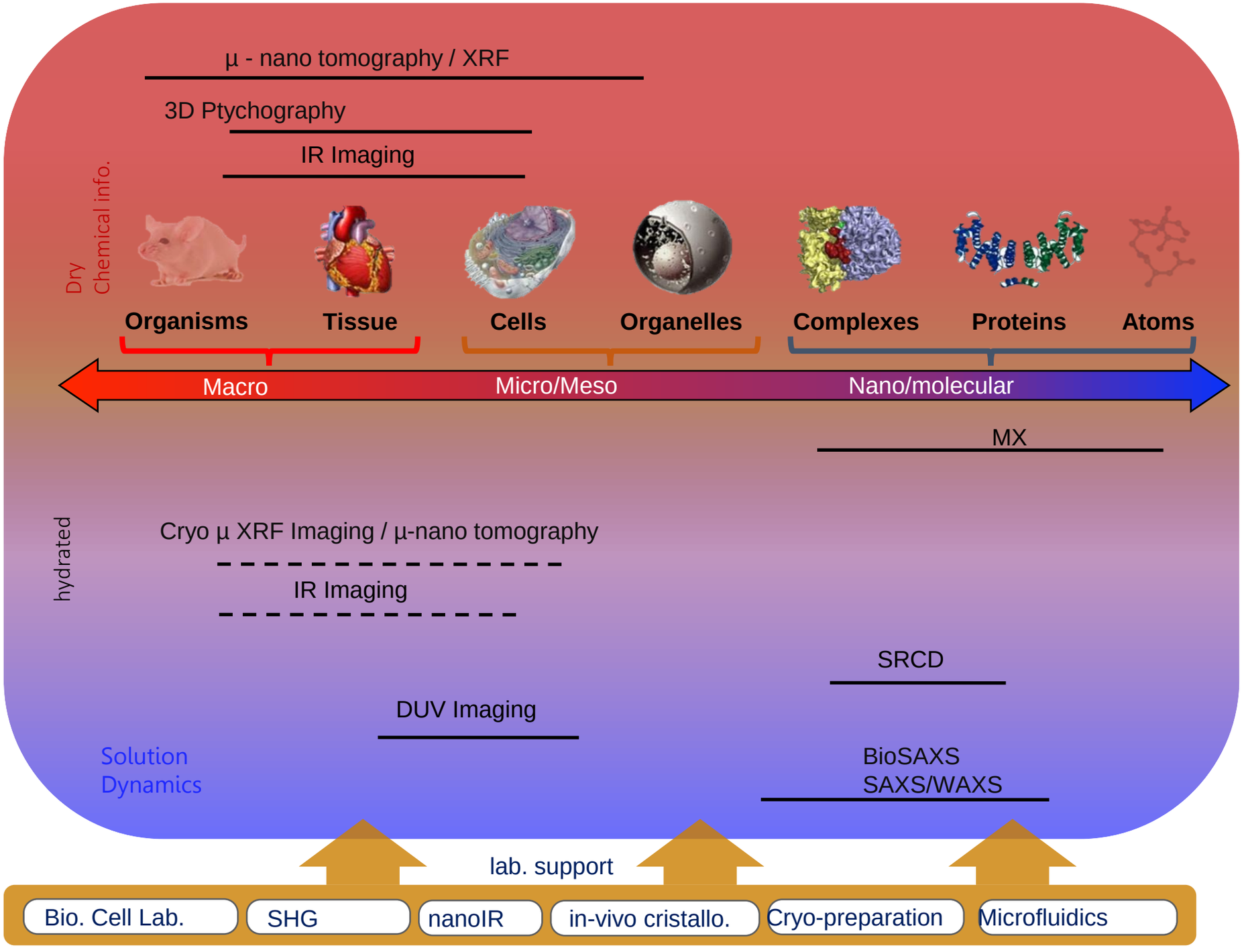
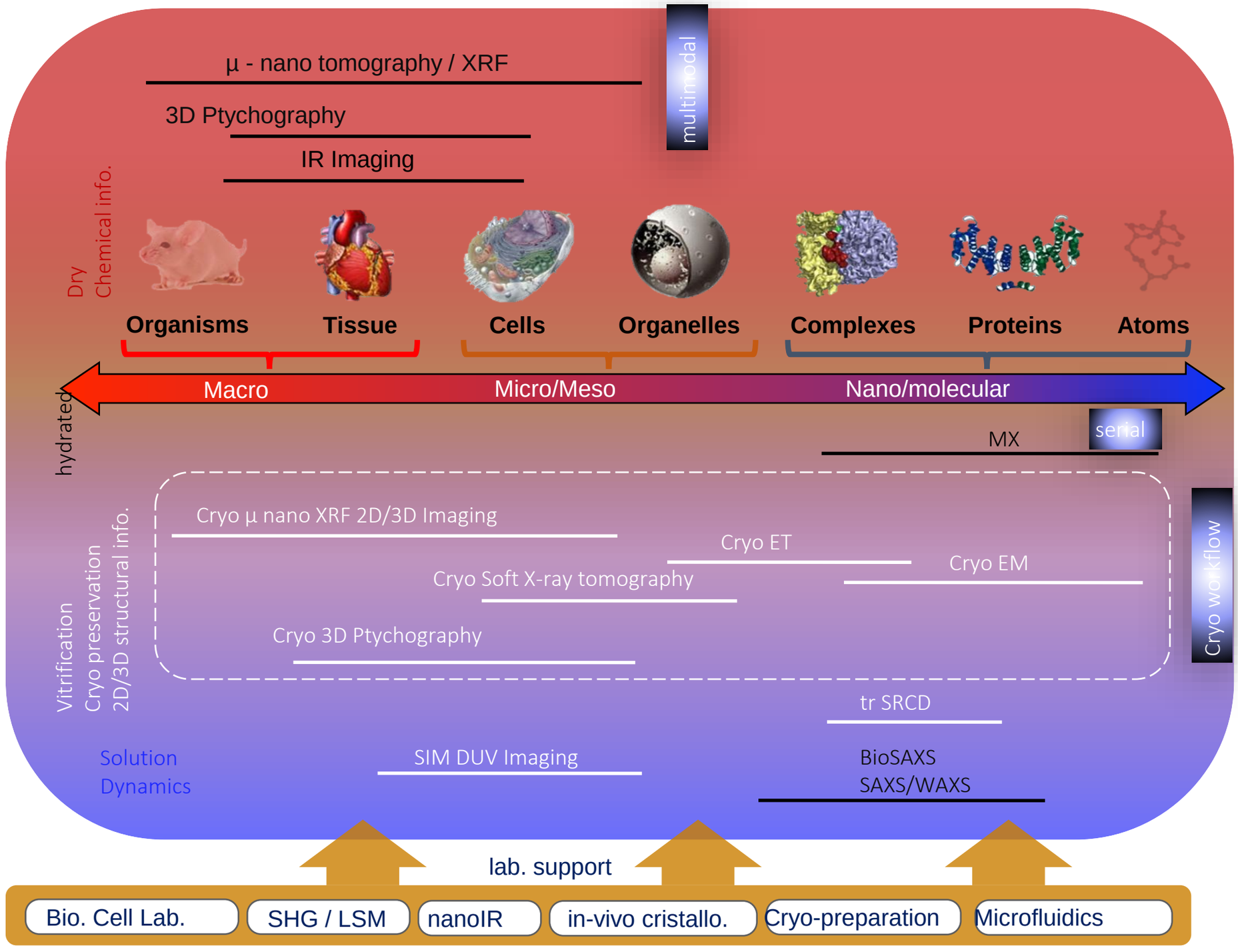


Figure 25: Comparison of the transverse beam profiles of SOLEIL and SOLEIL upgrade baseline lattice in a short straight section.






μ - nano tomography / XRF
3D Ptychography
IR Imaging
Dry
Chemical info.
Organisms
Tissue
Cells
Organelles
Complexes
Proteins
Atoms
Macro
Micro/Meso
Nano/molecular
MX
hydrated
Cryo μ XRF Imaging / μ-nano tomography
IR Imaging
SRCD
DUV Imaging
Solution
Dynamics
BioSAXS
SAXS/WAXS
lab. support
Bio. Cell Lab.
SHG
nanoIR
in-vivo cristallo.
Cryo-preparation
Microfluidics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MX
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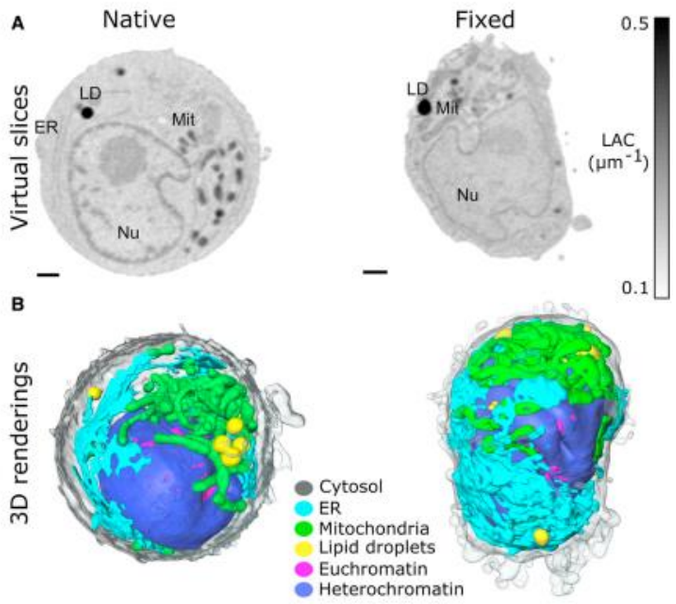
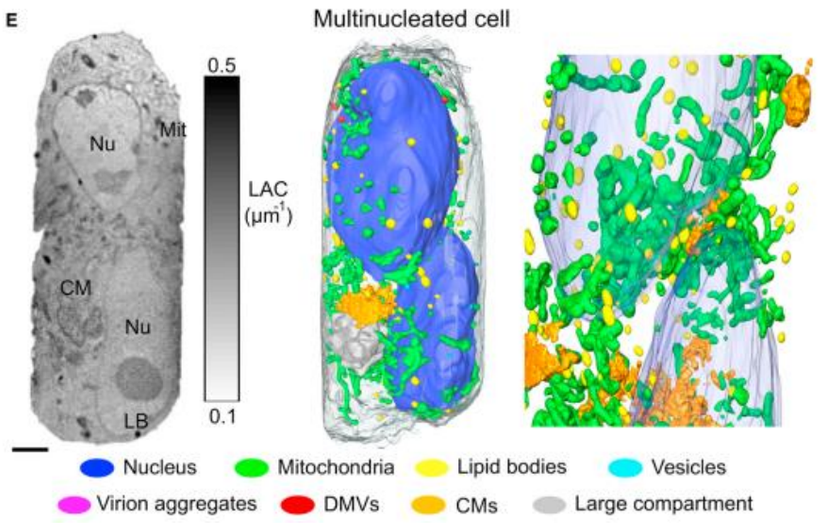
Cryo μ nano XRF 2D/3D Imaging
Cryo ET
Cryo EM
Cryo Soft X-ray tomography
Cryo 3D Ptychography
Vitrification
Cryo preservation
2D/3D structural info.
Cryo workflow
tr SRCD
Solution
Dynamics
SIM DUV Imaging
BioSAXS
SAXS/WAXS
lab. support
Bio. Cell Lab.
SHG / LSM
nanoIR
in-vivo cristallo.
Cryo-preparation
Microfluidics

Filling the resolution gap between imageries and structures

Organisms **Tissue** **Cells** **Organelles** **Complexes** **Proteins** **Atoms**

Soft x-ray tomography and Cryo-SIM





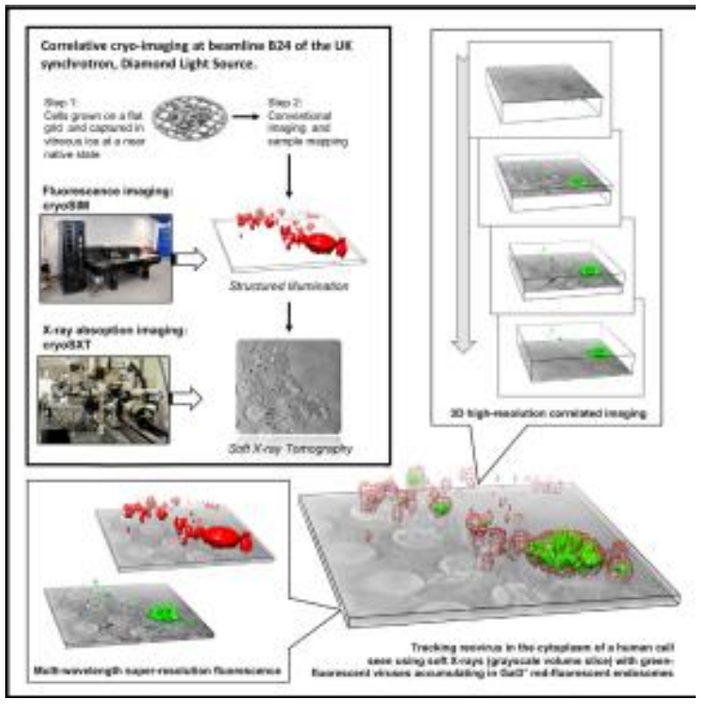
Weinhardt et al, 2021, ALS

Cryogenic Soft X-ray tomography :
A high throughput method
Full field imaging with good resolution
Penetration depth of soft X-rays ‘on the same scale’ as size of eukaryotic cells.
Water window : segmentation based on absorption contrast.

Low dose – identify ROI for subsequent of consequent ‘super resolution methods’ :
Cryo-SIM, Cryo-ET
Available at ALBA and DLS.

Upgraded storage rings -
Will gain little from high brilliance sources.

But possible extension of fiducial marker systems and cryogenic conditions to Cryo-ET (FIB-SEM preparation) and hard X-ray fluorescence (Harkiolaki et al, Nature protocols).

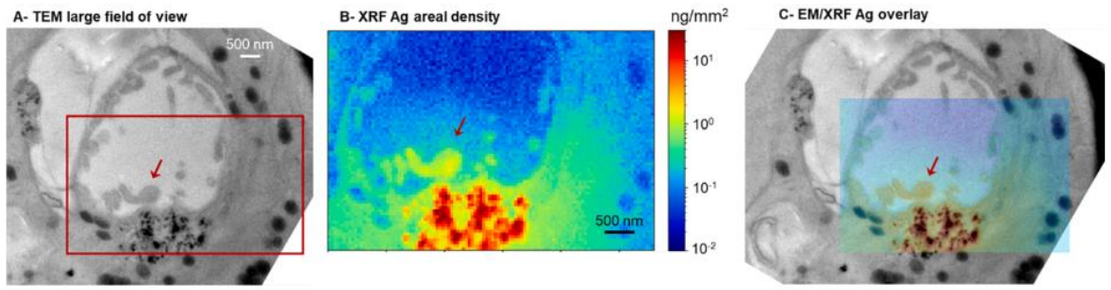


Kounatidis et al, Cell 2020

Cryo-SIM, similar sample thicknesses to sXRT, short image acquisition times.

Hard X-ray fluorescence microscopy and cryo-electron tomography

Advantages of scanning hard X-ray fluorescence microscopy : High spatial resolution, elemental specificity, high dynamic range, high sensitivity (for physiological concentrations), penetration depth.



Tardillo-Suarez et al, JSB 2021

- Correlative XRFM and ET.
- ESRF ID16 and Tecnai G2 spirit BioTwin at 120 kV.
- Fixed and embedded samples.
- EM grids 500 microns x 50 nm thickness.
- ROI selection 'roughly' found by optical microscopy.
- Possibility for workflow using cryo-protected samples and need for improved methods of sample transfer markers (nanoparticles etc) sensitive to all 'correlative' methods.
- Stronger sources will give increased speed and capacity to capture 'rare events'.

One main advantage of a single-cell, imaging-based analysis method compared to a bulk quantification approach is the possibility to identify specific events that occur rarely, but that are crucial to completely understand a biological process. Moreover, an imaging method can also dissect a whole process, by visualizing the whole cascade of events. In

Innovative labelling chemistry – Single Core Multimodal Probes for Imaging.

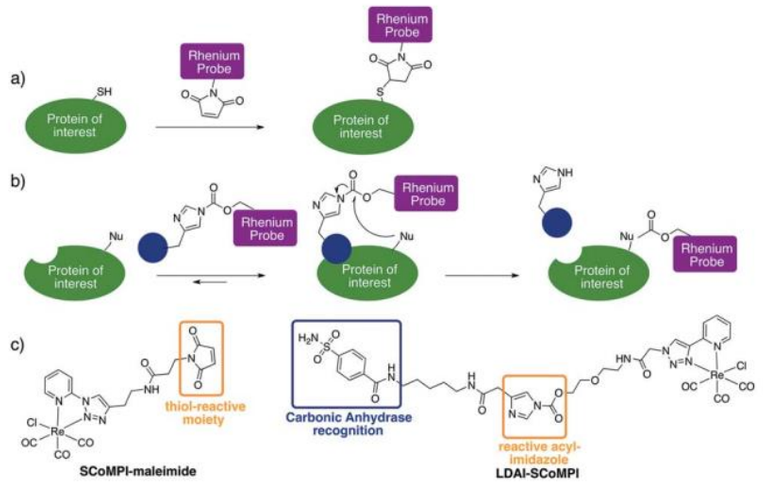
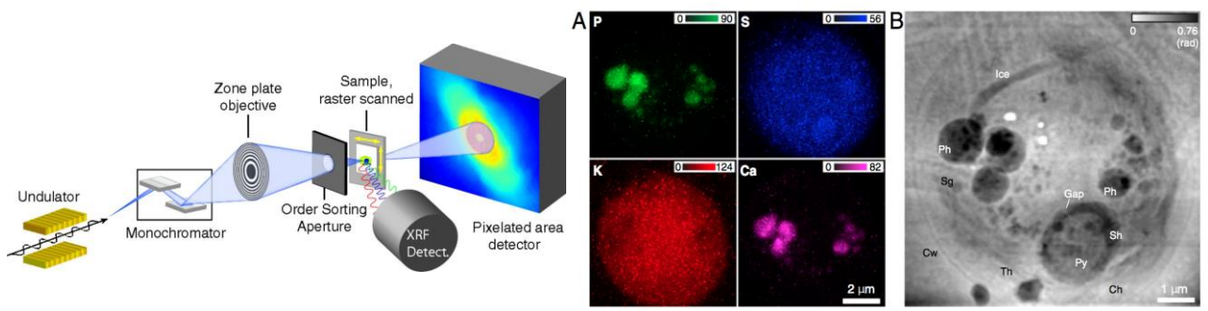


Fig. 1 Labeling of proteins with SCoMPI for X-ray fluorescence imaging. (a) Labeling of an exogenous protein using the thiol maleimide reaction. (b) Labeling of an endogenous protein using the "Ligand-Directed Acyl Imidazole" chemistry. The blue circle represents a ligand of the protein. (c) (left) Structure of SCoMPI-maleimide for the *in vitro* labeling of homeodomains and (right) structure of LDAI-SCoMPI for the labeling of endogenous carbonic anhydrases.

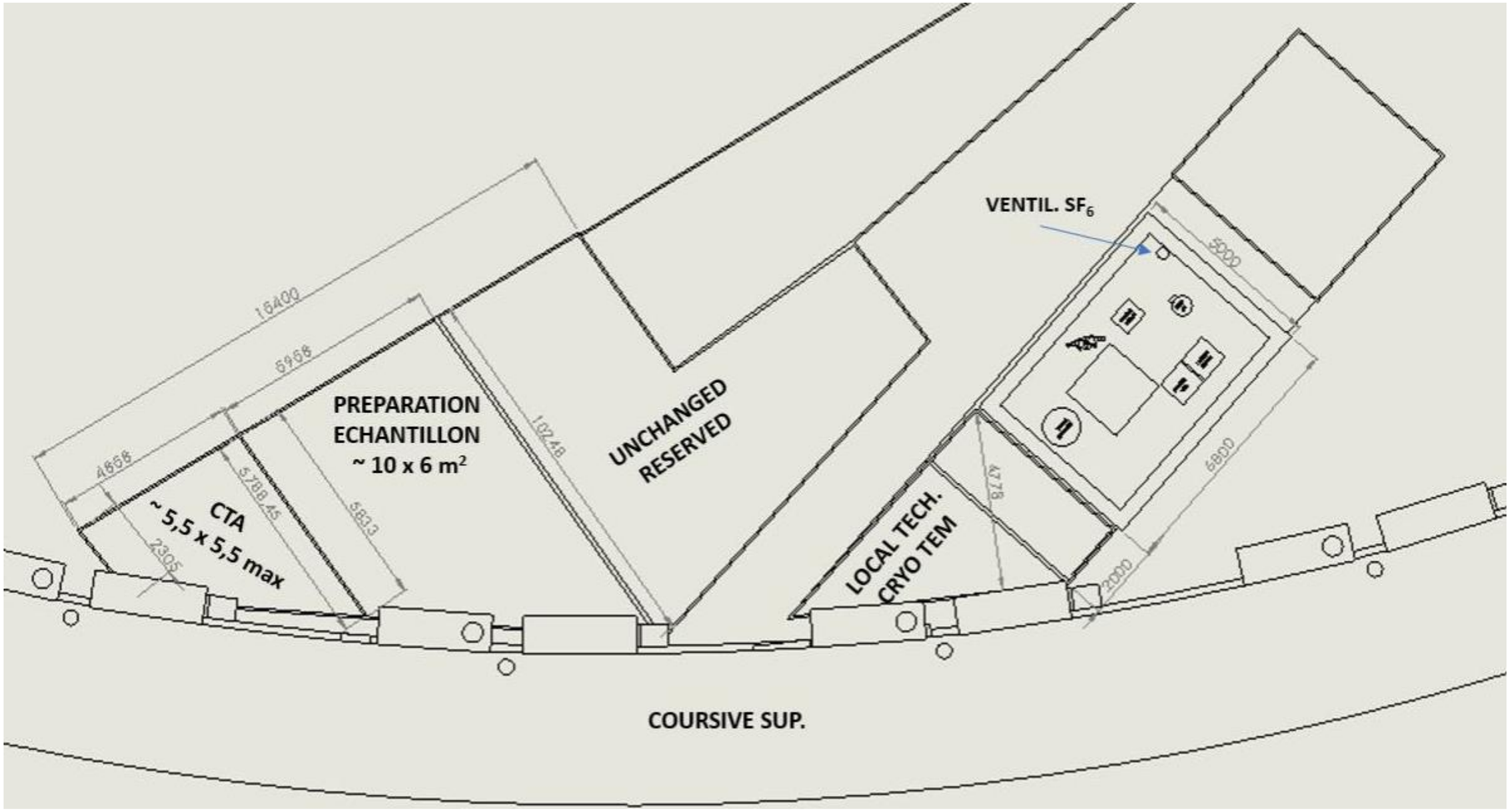
From Policar et al, Chem. Sci. 2018

Combining ptychography with XRFM – proof of principle at APS.



Deng et al 2015 PNAS.

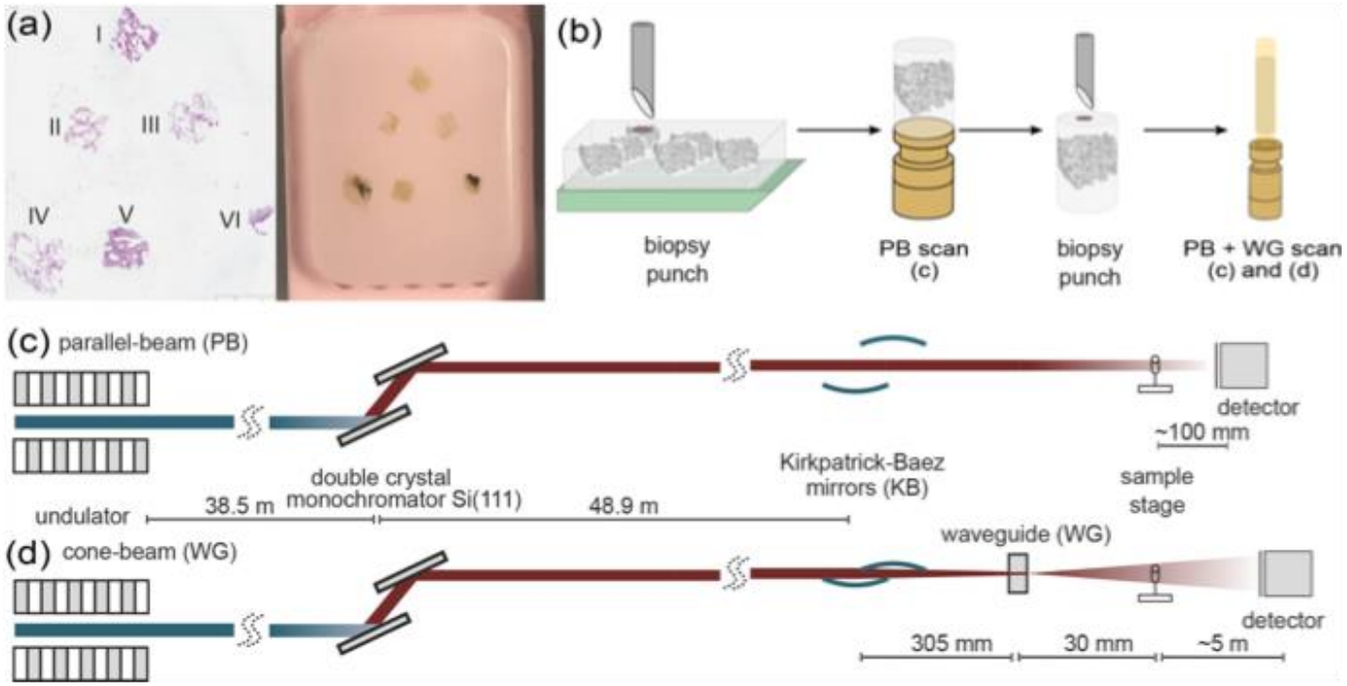
Proposed site for 300 kV Cryo-EM at SOLEIL

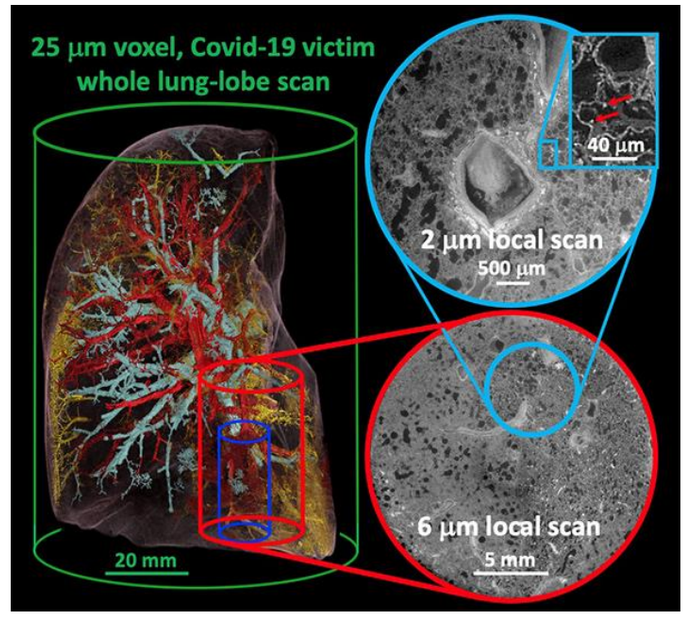


Operation planned for summer 2023.

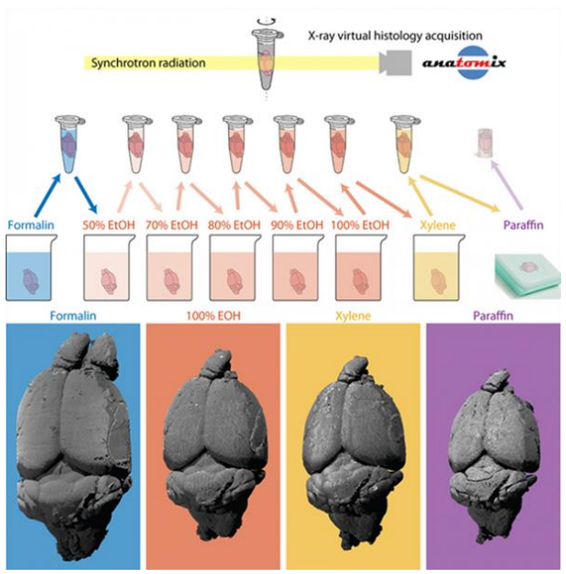
Studying larger objects : 3D virtual histology by hierarchical phase contrast tomography, from high brilliance sources.

- Eckermann et al, eLIFE 2021, DESY.
- Walsh et al, Nature Methods 2021, ESRF-EBS





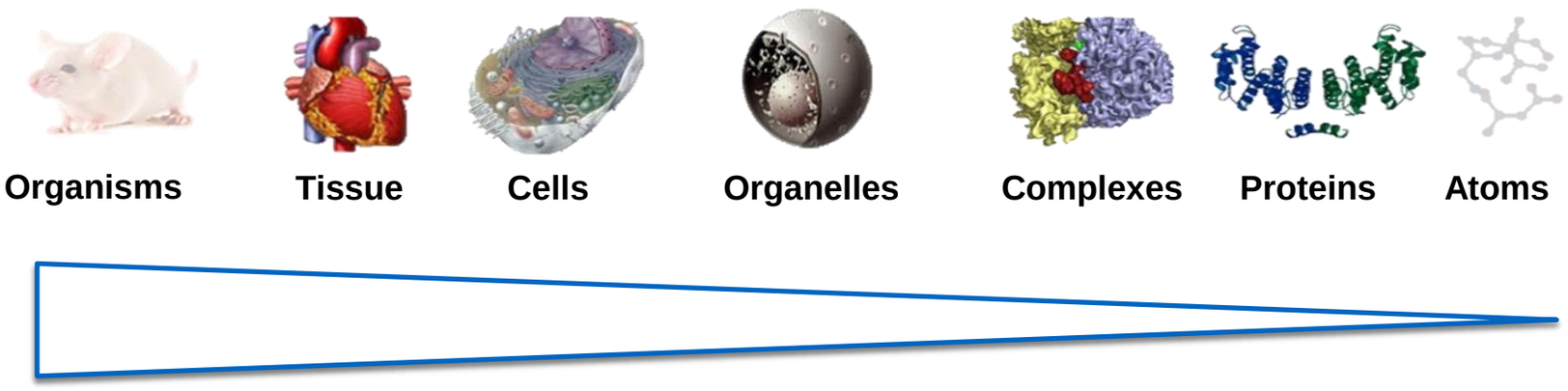
Zoom ‘in situ’ by changing beamline or detector optics, unstained large sample sizes.



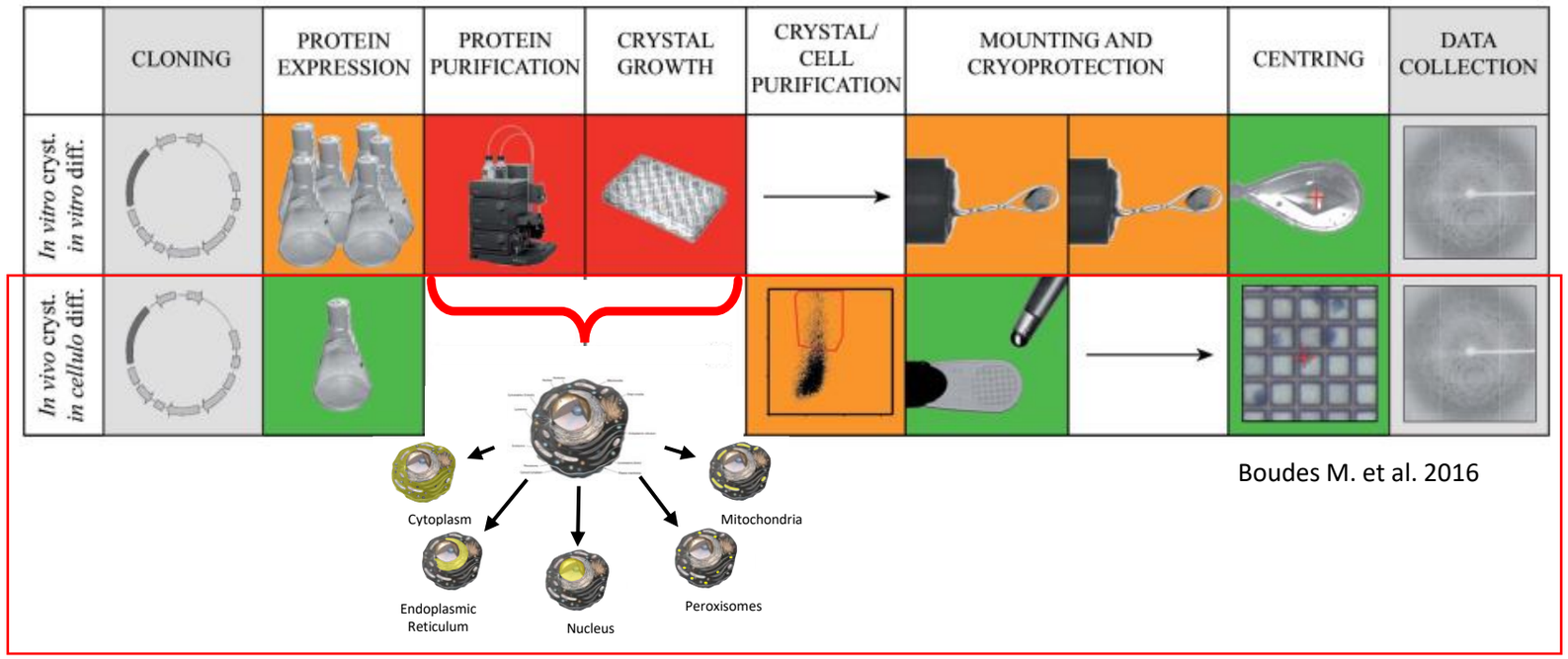
Rogers et al. Journal of Neuroscience methods, 2021

Careful acquisition procedures to control radiation dose and beam heating.
Fixing procedures can change both size (locally and globally) and contrast of reconstructed images. Interpretation can be corrected by atlas of sample preparation effects.

Developing tools and methods for a multimodal approach



Why is *In vivo* protein crystallography attractive ?



Boudes M. et al. 2016

To what extent can we turn cells into protein crystal factories ?

Understand the basic underlying principles

Set up a workflow for routine *in vivo* crystallization

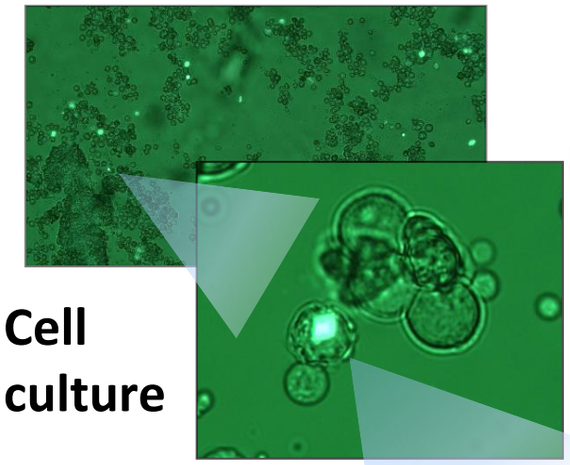
Requires a model protein : XPA

An engineered fluorescent protein that spontaneously crystallizes in human cells.

XPA (PDB 4P76) : variant of red-green fluorescent protein, easy to detect in cells. Tsutsui et al, Molecular Cell 2015

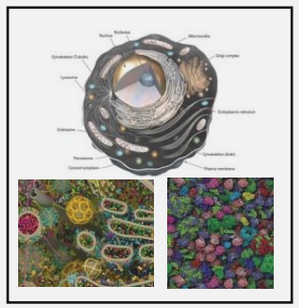
Crossing length scales to understand the process

Molecular Biology/ cell b



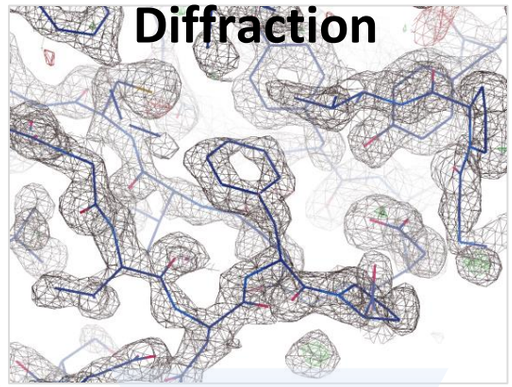
Cell culture

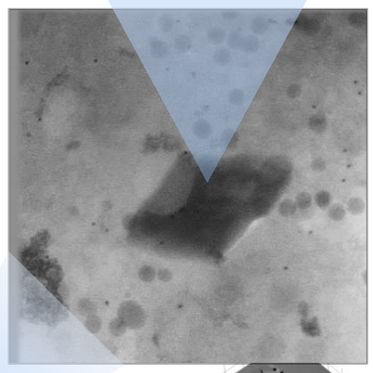
XPA

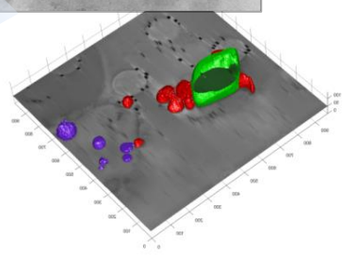


HEK293

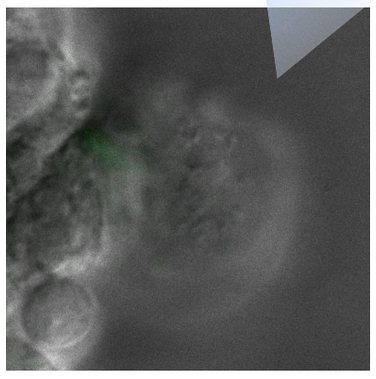
X-ray Diffraction





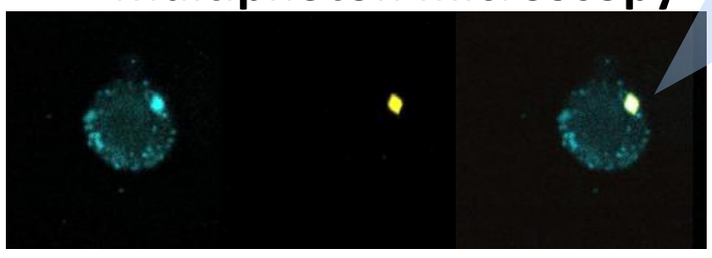


Cryo Soft X-Ray Tomography



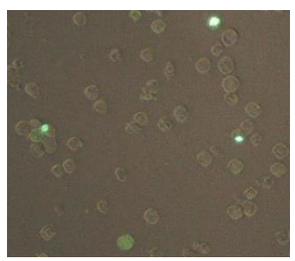
Fluorescence and DIC microscopy

Multiphoton microscopy

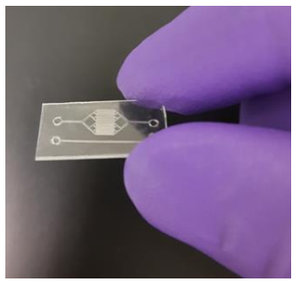


Fluorescence SHG merge

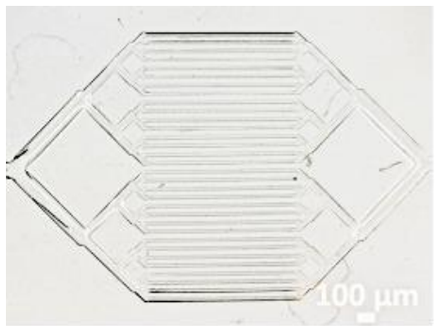
Proof of concept using microfluidic chip, multimodal approach and XPA as model



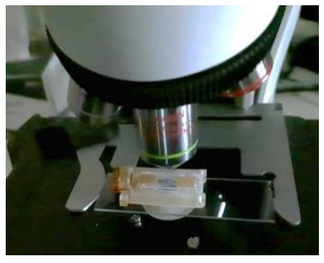
XPA transfected human cell culture

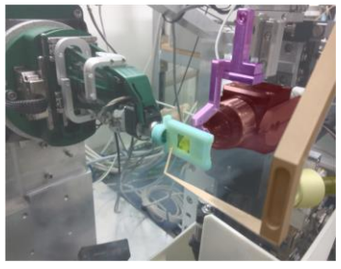


Multiple channel microfluidic COC Device.
Low x-ray background.
Multimodal imaging compatible



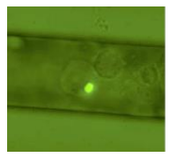
Fluorescence, DIC, SHG…. microscopies



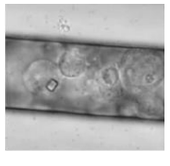


Serial X-ray diffraction

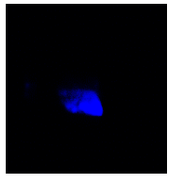
Fluorescence

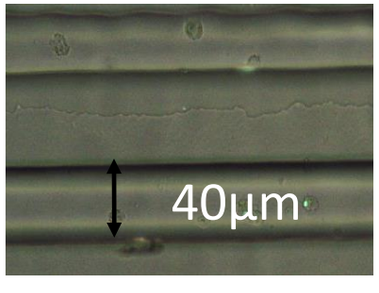


Differential Interference Contrast

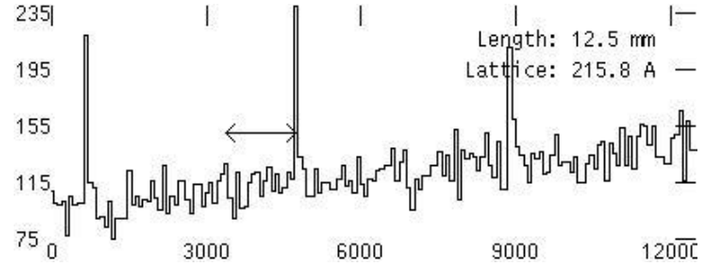


Second Harmonic Generation





2-7 µm sized Xtals in cells.



100 images, 1° spot observed up to 4 Å

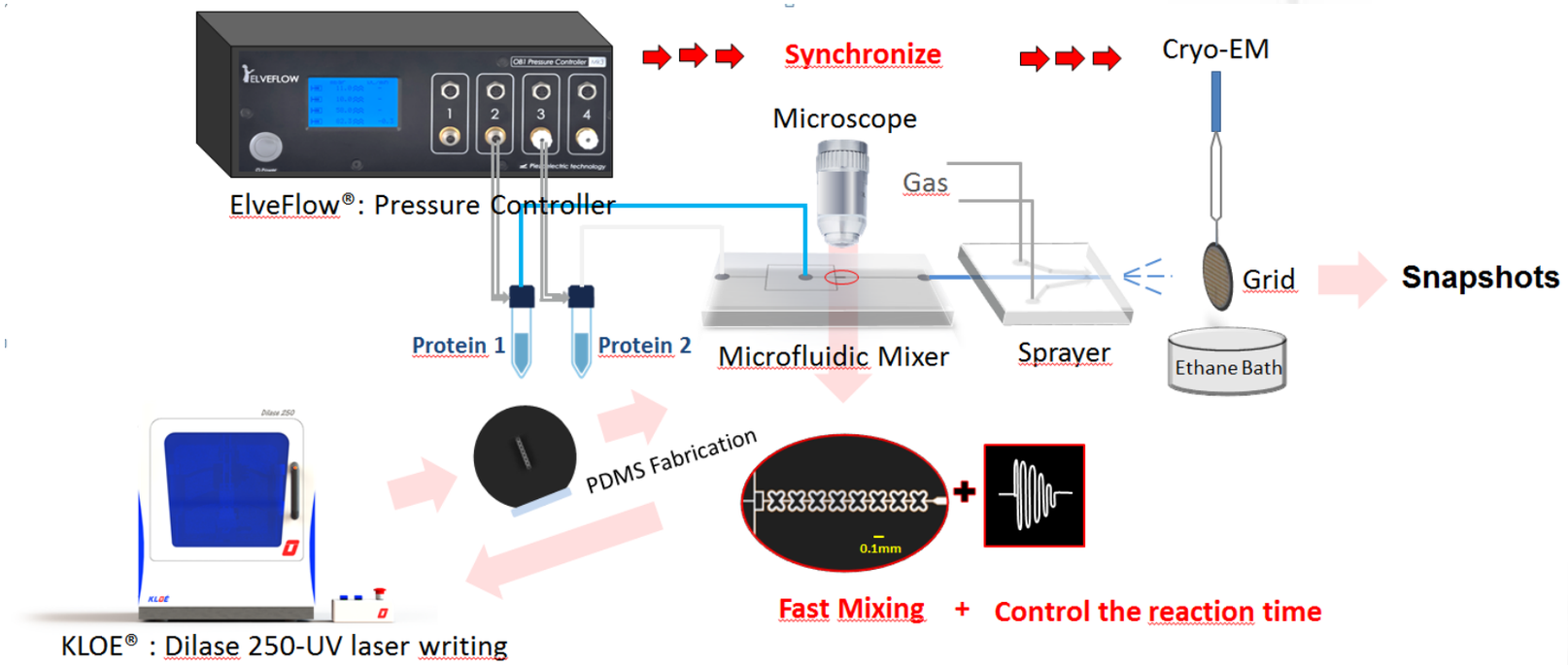
Data collection angle -30° <-> +30°

Fast mixers for time-resolved cryoEM

PROJET FINANCÉ PAR L'ANR - PROJECT FUNDED BY THE ANR

Collaboration with the team of Dr. Yves Mechulam, Ecole Polytechnique.

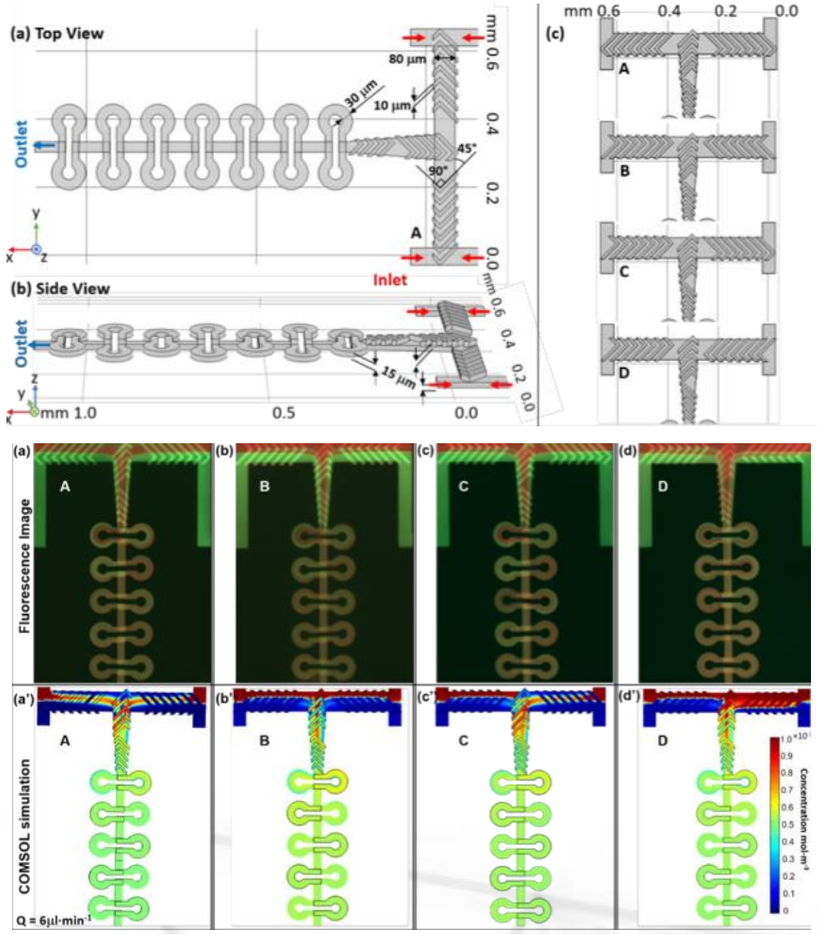
- Reduced sample volume required
- Better control of sample preparation
- Access to transient intermediates

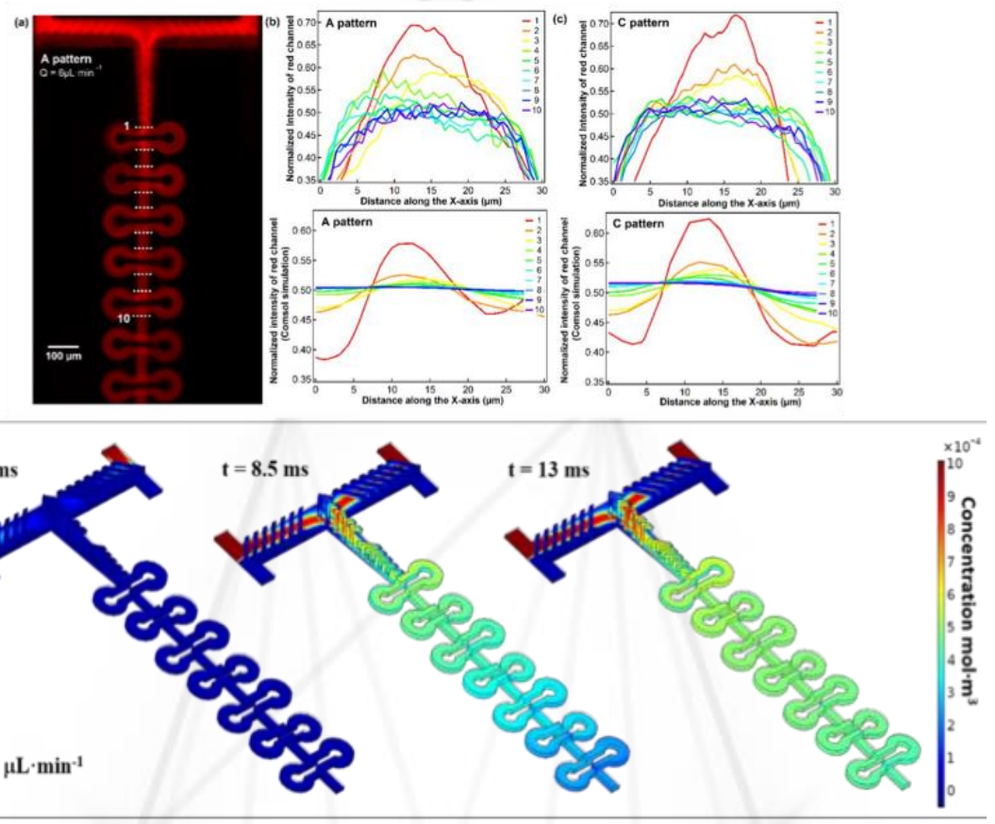


Yuan-Yuan Liao

Fast mixers for time-resolved cryoEM (Liao et al 2021)

- Mixing time of *ca.* 30 ms achieved with simple pressure systems
- Micro-spray under development (for em grids / cryo-plunge)
- Total volume 3.6 nL, low flow rate (24 μL per minute)





SHB proposed by Stroock et al 2003.

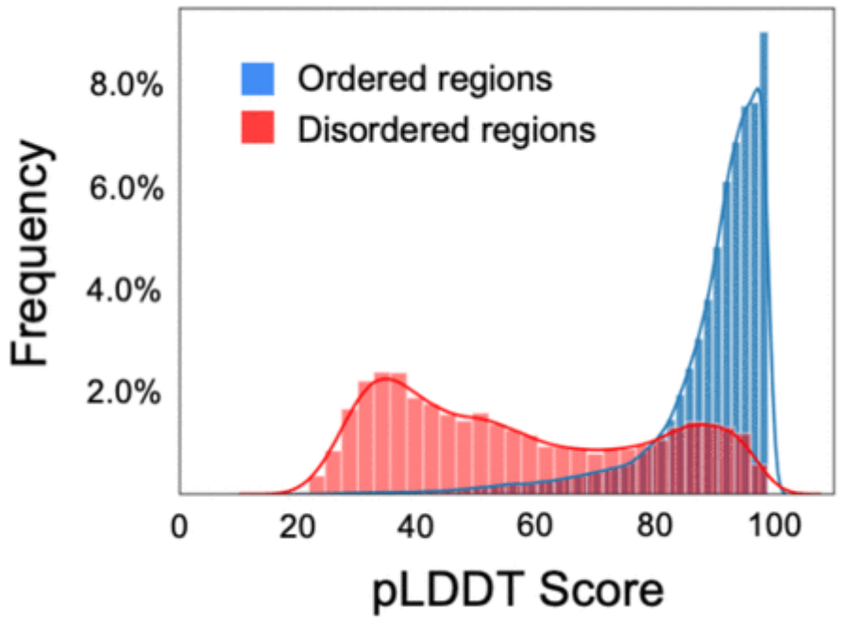
Other combinative approaches exist – Spotiton (Carragher et al JSB 2018), Kontziampasis et al IUCRJ 2019 and Ravelli et al (Nature Communications 2020)..

Coupling modelling and measurement.

- Predictive power of machine learning methods (Alphafold2, RoseTTAfold etc) is being learned by the community :
  - Model for X-ray structure solution by MR.
  - Improvement of low resolution crystallographic structure models making it easier to combine Cryo-EM and X-ray?

Future impact in Integrative Structural Biology.

- Bridging high resolution structure with imaging methods – multimeric complexes, disordered regions.
- Several studies in BioRXiv suggest that pLDDT indicative of order / disorder rather than confidence (but perhaps can suggest ‘conditionally foldable’ regions).
- Coupling of models with validation by solution techniques and NMR.



From Binder et al, BioRXiv 2021

What will the ultra high brilliance synchrotron sources bring to integrative structural biology?

Organisms **Tissue** **Cells** **Organelles** **Complexes** **Proteins** **Atoms**

Synchrotron and Lab based Methods : Structure

Today:

- Measure high resolution molecular structures in single (MX) or multiple (Cryo-EM) configurations under cryogenic conditions
- Begin to look at structures at medium resolution in a cellular environment (Cryo-ET), or at very low concentrations (in cellular NMR)

SOLEIL Upgrade Opportunities:

Maintain access and expertise in X-ray diffraction methods whilst making new methods user friendly (*in vivo*, serial crystallography….)

Extend to time domain for ordered structures. (Multi-) pink beam diffraction at room temperature. Complementarity with NMR.

Improve access and coupling to other structural methods, putting them in a cellular context either directly (pushing limits of X-ray or electron tomography, Plitzko et al Nat Meth 2019) or indirectly by coupling with imaging / spectroscopies.

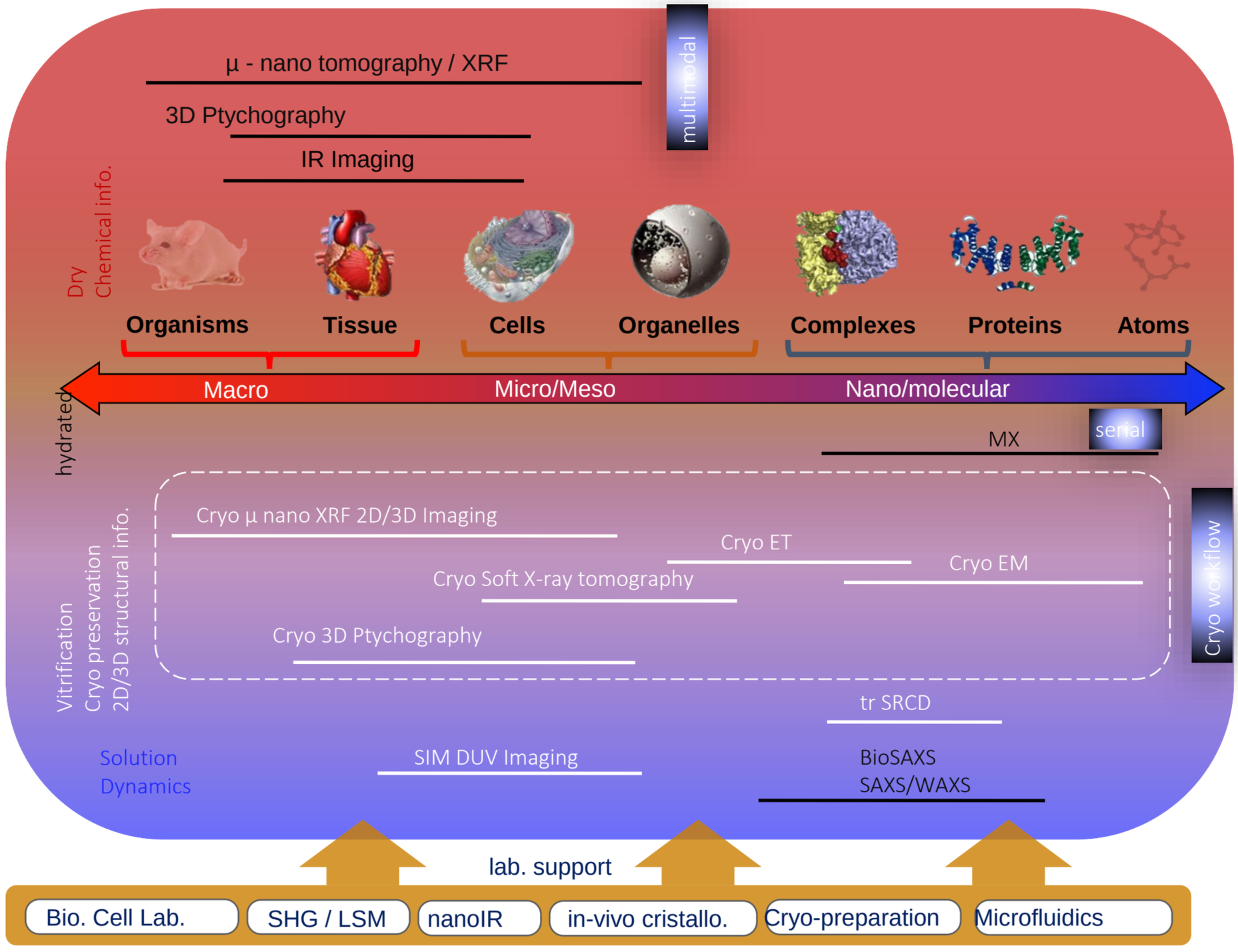
Synchrotron and Lab based Methods - Imaging

Today :

- (Hyperspectral) imaging in 3D at different spatial resolutions (µm to 10's of nm) from live or preserved (paraffin, chemically fixed, cryo-protected) cells.
- Super-resolution fluorescent microscopy

SOLEIL Upgrade Opportunities :

- Coherent imaging methods. Higher spatial resolution. Better elemental sensitivity. Faster collection time hence larger cohort of samples and possibility to study variation. Energy resolved detectors. Super-resolution techniques? Correlative methods and putting information into its correct context. Cryogenic workflow – transfer (of samples and information) between imaging modalities – needs major investment in data storage, computer power and effort in data analysis / visualization.


multimodal
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Cryo EM
Cryo Soft X-ray tomography
Cryo 3D Ptychography
Vitrification
Cryo preservation
2D/3D structural info.
Cryo workflow
tr SRCD
Solution
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SAXS/WAXS
lab. support
Bio. Cell Lab.
SHG / LSM
nanoIR
in-vivo cristallo.
Cryo-preparation
Microfluidics

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- SOLEIL beamlines ANATOMIX, NANOSCOPIUM, DISCO, SMIS, PROXIMA 1, PROXIMA 2a and SWING
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