Advancements in Biological Sciences at the European Synchrotron

May 26th 2025

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Head of Structural Biology Group

ESRF 1994 - 2024

A NEW BIOLOGY FOR THE 21ST CENTURY

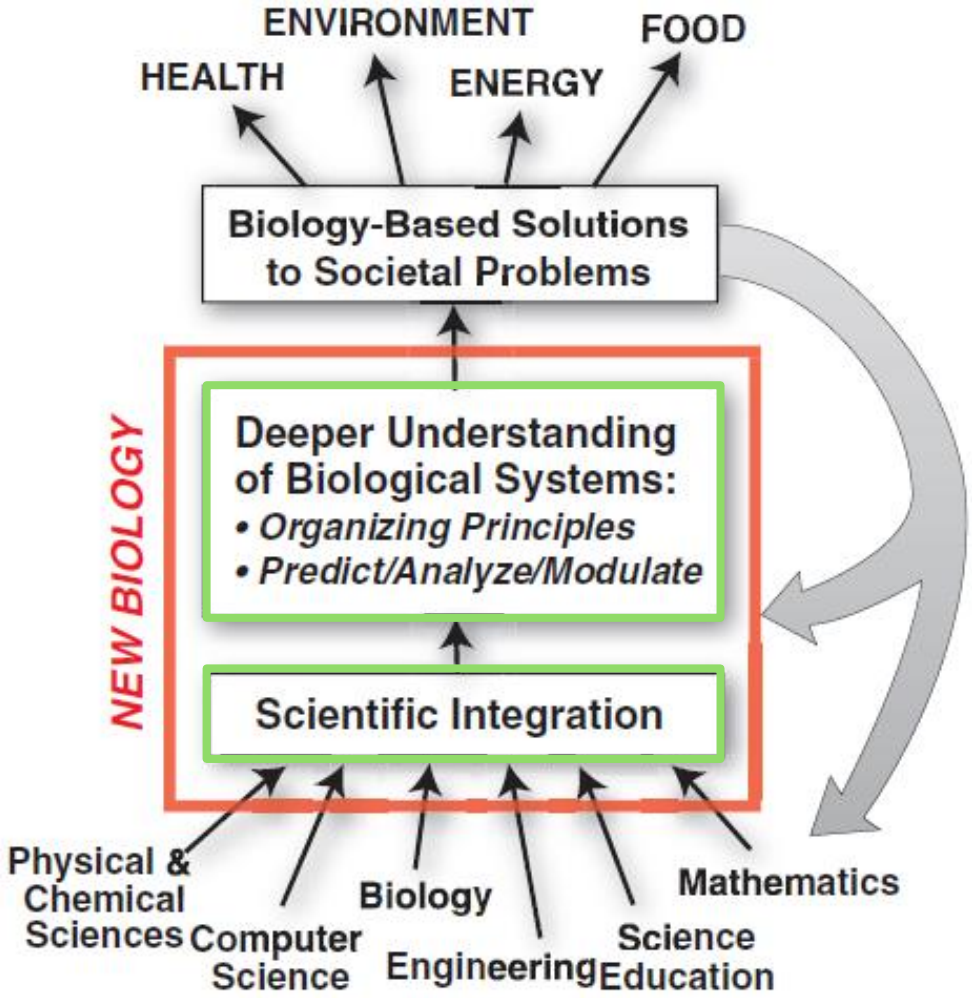
As never before, advances in biological sciences hold tremendous promise for surmounting many of the major challenges confronting the world

Deepen fundamental research

Understanding of the organizational principles of complex biological systems to solve large-scale problems

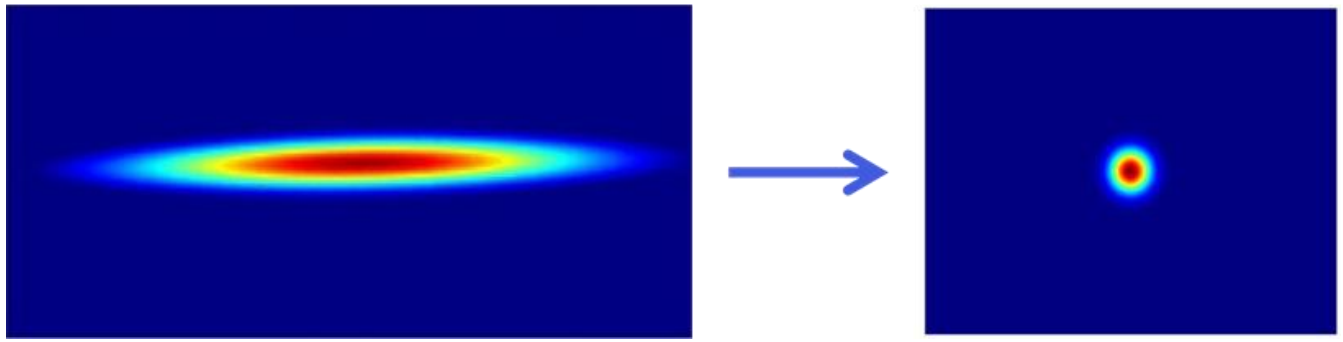
Integration across disciplines

(Re-)integration of powerful technologies along with new concepts and methods from physical sciences, mathematics, computational sciences and engineering



SOURCE: Committee on a New Biology for the 21st Century. The National Academies of Sciences, Engineering, and Medicine (USA)

CARRYING THE SPIRIT FORWARD AT ESRF, 4TH GENERATION SINCE 2020



ε_x ~ 4000pm

ε_x ~ 133pm

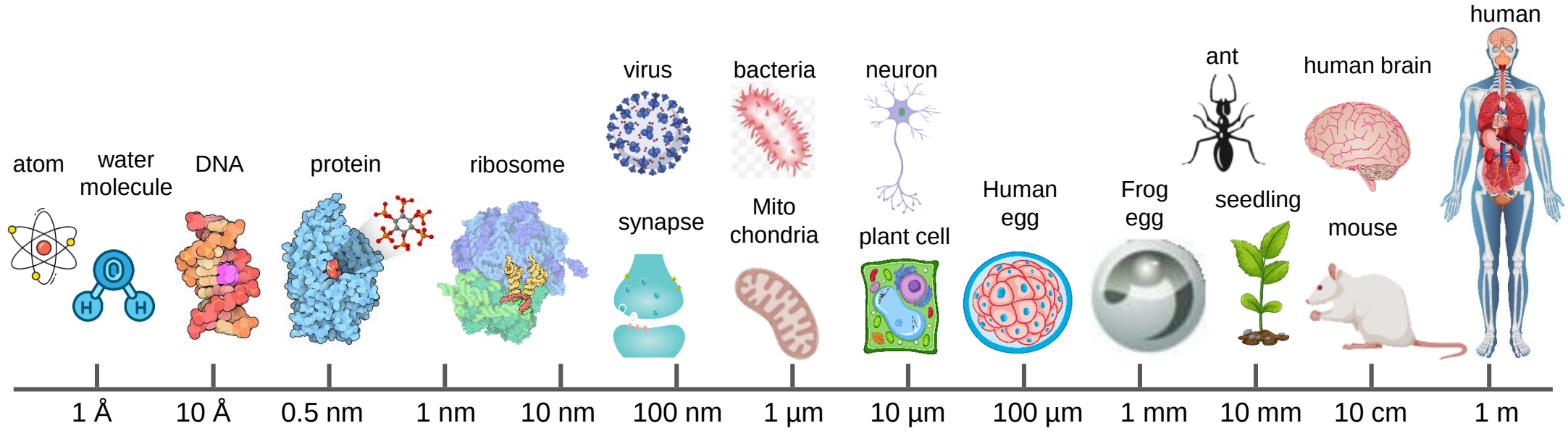
× 30 Trans. Coherence

× 100 Brilliance

÷ 100 Hor. Emittance

Beam sizes from nm to 320 mm	Greater coherence-based approaches	Higher throughput with dose tolerance	Higher energies with higher fluxes	Better sensitivity Artifact mitigation
X-Ray Microscopies Larger field of view	CDI, Phase Contrast, Holography, XPCS *High quality Optics*	Faster dynamics Time-resolved experiments, *cryo…*	Larger penetration Low dose *in vivo* imaging, in situ, *operando*, extreme (T,P,H), …	Better detection limits, noise reduction

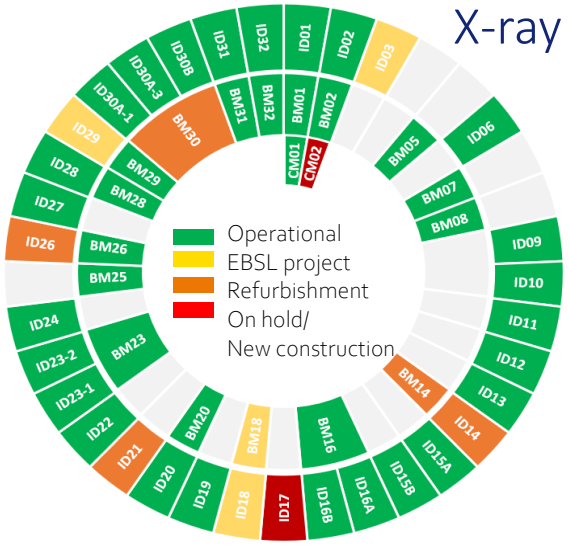
SYNCHROTRON TECHNIQUES FOR BIOLOGY RESEARCH



X-ray diffraction/scattering

X-ray spectroscopy

X-ray imaging



Courtesy: Delphine Pauwels

STRUCTURAL BIOLOGY @ THE EUROPEAN PHOTON & NEUTRON CAMPUS

European Photon & Neutron Science Campus

EMBL
European Molecular Biology Laboratory

ESRF
European Synchrotron Radiation Facility

IBS
Institut de Biologie Structurale

ILL
Institut Laue-Langevin

PSB
Partnership for Structural Biology

PSCM
Partnership for Soft Condensed Matter

THE PARTNERSHIP FOR STRUCTURAL BIOLOGY

The primary mission of the PSB is to foster integrative structural biology.

Brings together

- 350 scientists
- 45 Postdocs
- 75 PhD students
- Reviewed every 3 years by an external SAB

ACCESS TO TECHNOLOGICAL PLATFORMS FOR INTEGRATED STRUCTURAL BIOLOGY

a unique portfolio of 26 technological platforms and sample preparation labs

Sample preparation and optimization

- Eukaryotic Expression Facility
- Cell Free Expression
- ESPRIT
- Deuteration Lab
- NMR Quality Control
- Protein Sequencing
- EM Quality Control

High-resolution structure

- HT crystallisation
- High-field NMR
- Neutron Diffraction

Supramolecular analysis

- SANS

Biophysical characterization

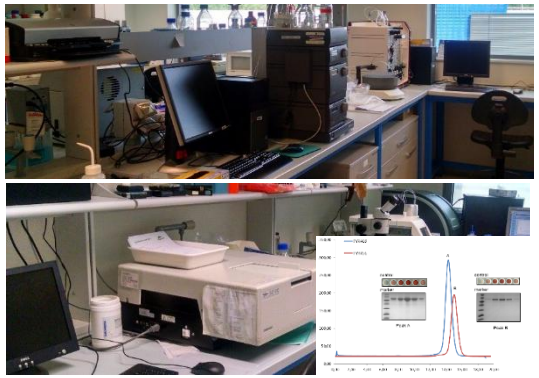
- MALS(SEC)
- AUC
- ITC
- SPR
- MST
- CD
- DLS
- Mass spectrometry
- Mass photometry
- spectroscopy

PROTEIN SUPPORT LABS

Users can have access and technical support to protein preparation and characterization prior to experiments

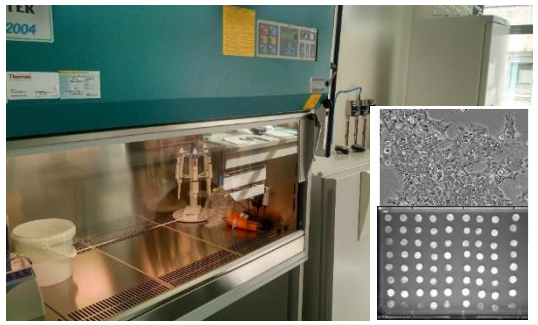
protein overexpression

Protein purification and biochemistry



Crystallisation & Cryocooling

Cell culture (mammalian, yeast)



https://www.esrf.fr/home/UsersAndScience/support-and-infrastructure/support-labs/protein-support-labs.html

THE STRUCTURAL BIOLOGY BEAMLINES AT THE ESRF

Structural information is essential to understand the underlying mechanisms, etiology and progression, which can lead to the development of more stable forms of proteins or drug compounds to modulate their function

With EMBL — X-RAY MACROMOLECULAR CRYSTALLOGRAPHY

- **ID30A-1**: fully automated 12.8 keV, 20-100 µm
- **ID30B:**
- **ID23-1:**

  } tunable 6-20 keV (2.0-0.62 Å), 20-50 µm
- **ID30A-3**: minifocus 12.9 keV (0.96 Å), 15 µm
- **ID23-2**: microfocus 14.2 keV (0.87 Å), 5 µm
- **EBSL8 (ID29)**: serial, nanofocus 10-25 keV (1.24- 0.5 Å), 2 µm

With ibs

- **BM07/FIP2**: tunable 7-15 keV, 50 – 250 µm*

With EMBL — SMALL ANGLE X-RAY SCATTERING

- **BM29**: 7-15 keV, 50 µm – 1.0 mm
  high-throughput, online size exclusion purification

With ibs EMBL — CRYO-ELECTRON MICROSCOPY

- **CM01**: 300 kV, single-molecule/tomography
- **CM02**: 300kV, single-molecule/tomography*

With ibs — COMPLEMENTARY METHODS

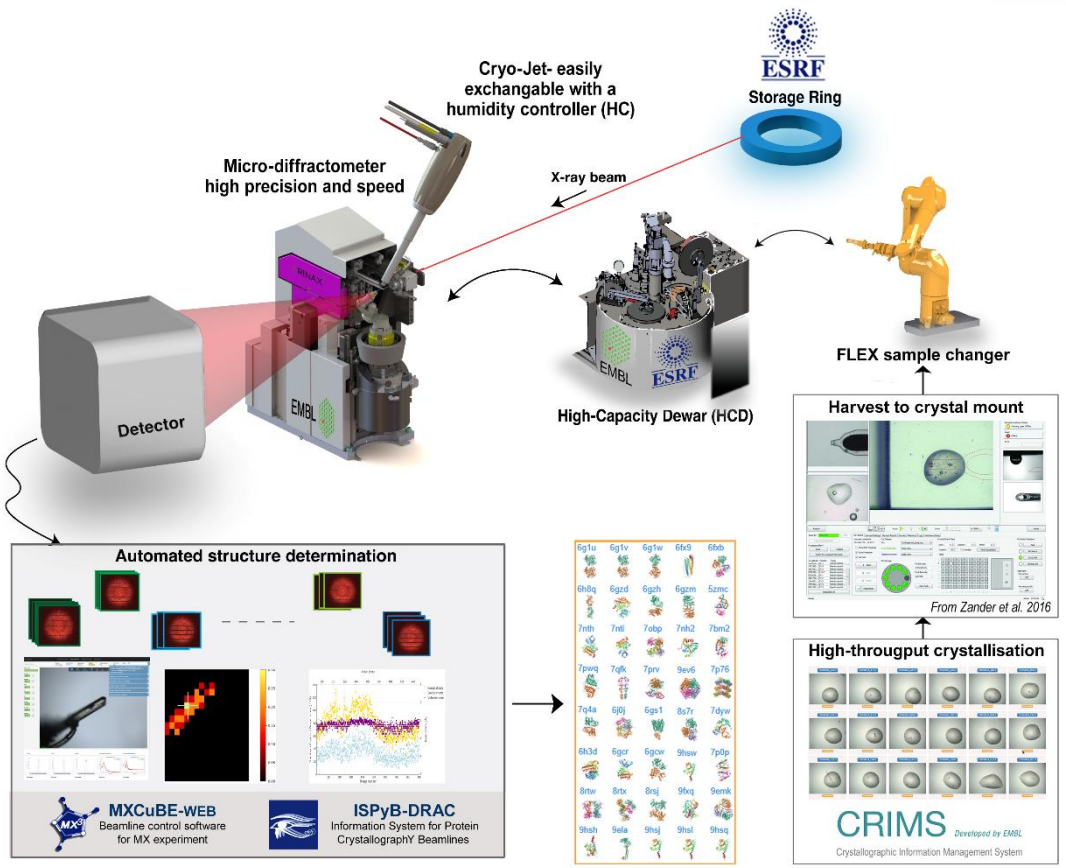
- **icOS**: *In crystallo* optical spectroscopy: UV/Vis absorption, fluorescence, Raman

With cea GRENOBLE

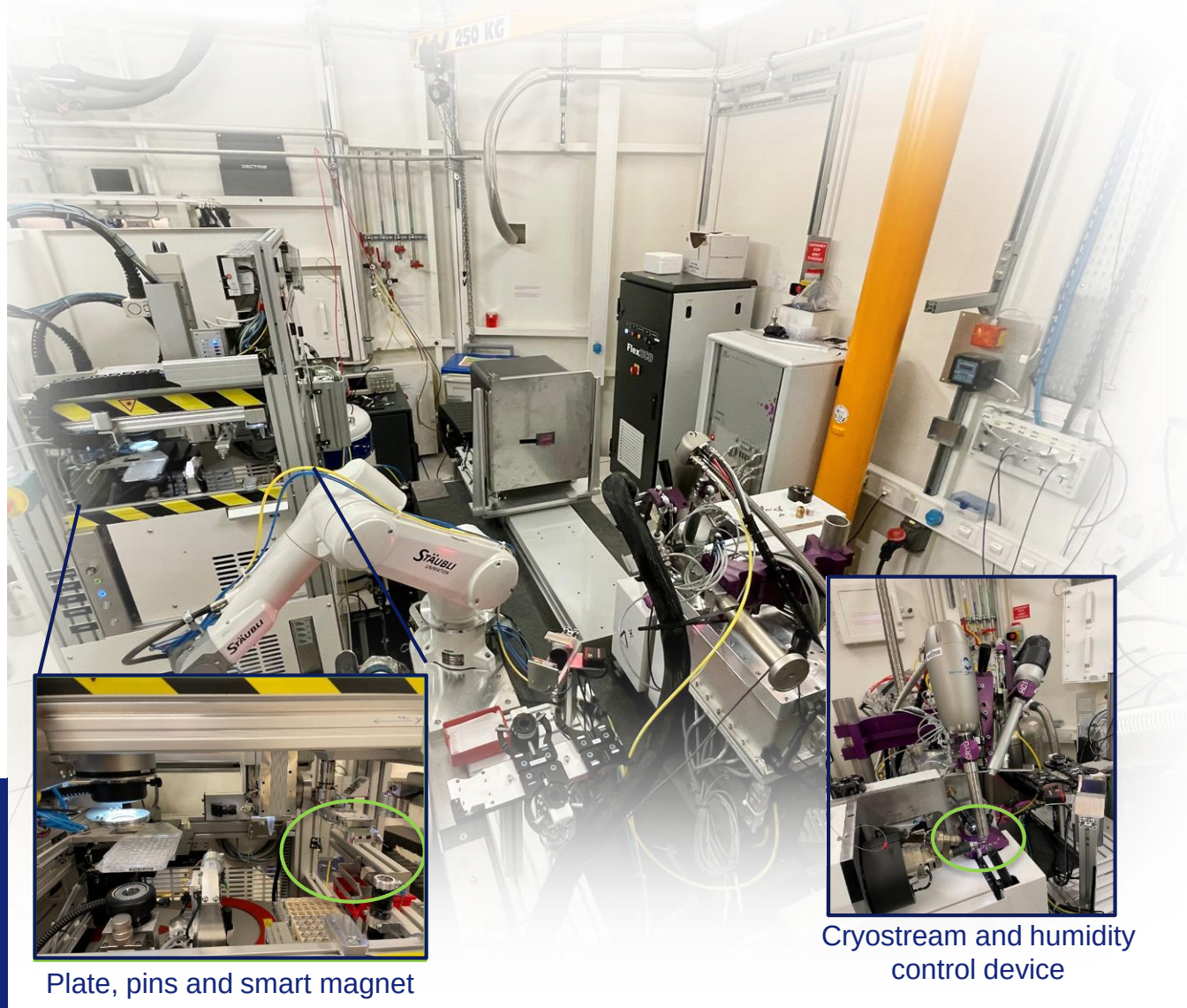
- **HPMX**: high-pressure crystal freezing 200-2000 bar, cryo-protectant free cooling, introduction of gases

***CRG**

BUILDING TOMORROW'S TOOLS FOR STRUCTURAL DISCOVERY: AUTOMATED CRYSTAL HARVESTING AND DATA COLLECTION PIPELINES



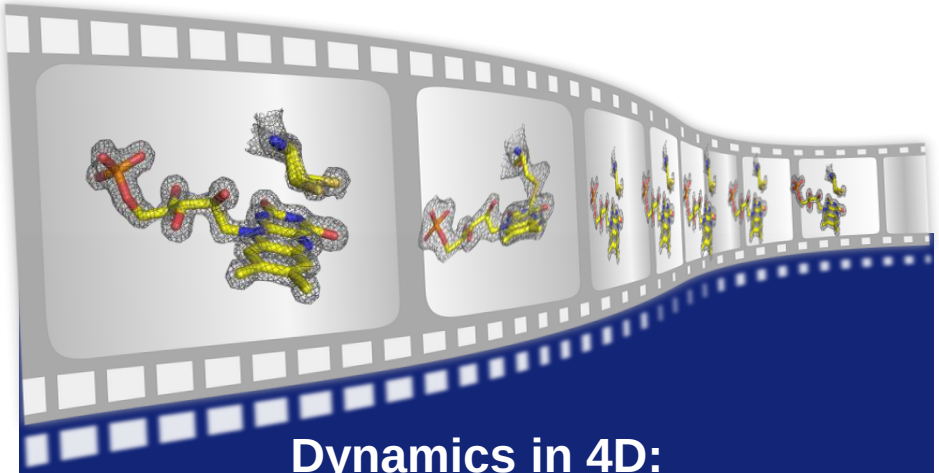
Structure-based compound screening
@ near physiological conditions:
from protein sample to ligand complexes



Plate, pins and smart magnet

Cryostream and humidity control device

Courtesy of M. Bowler (EMBL) & D. Nurizzo



Dynamics in 4D:

resolution, time, and temperature

MX, SAXS, cryo-EM

EBSL8 – ID29: SERIAL CRYSTALLOGRAPHY (SµX)

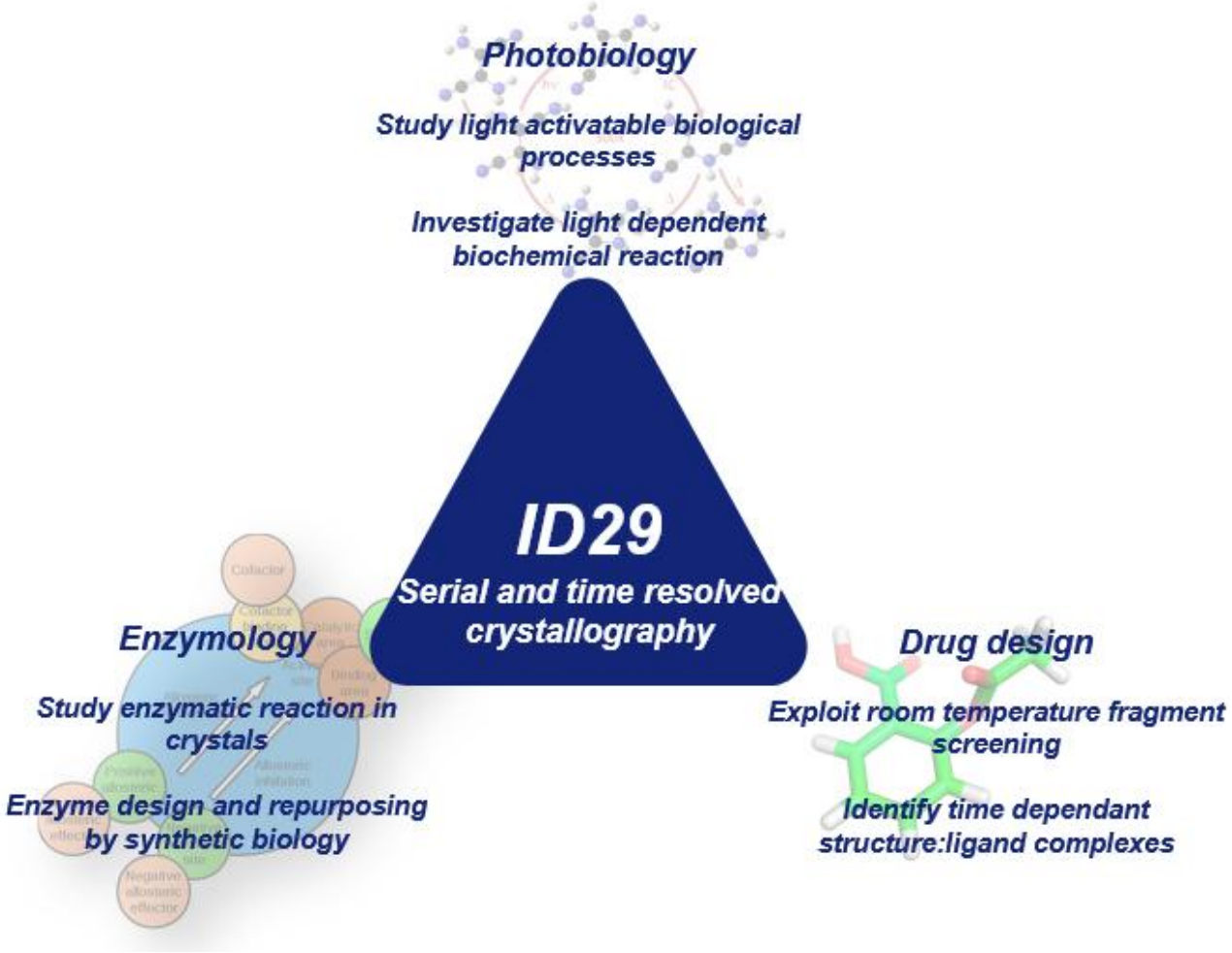
in microsecond time scale

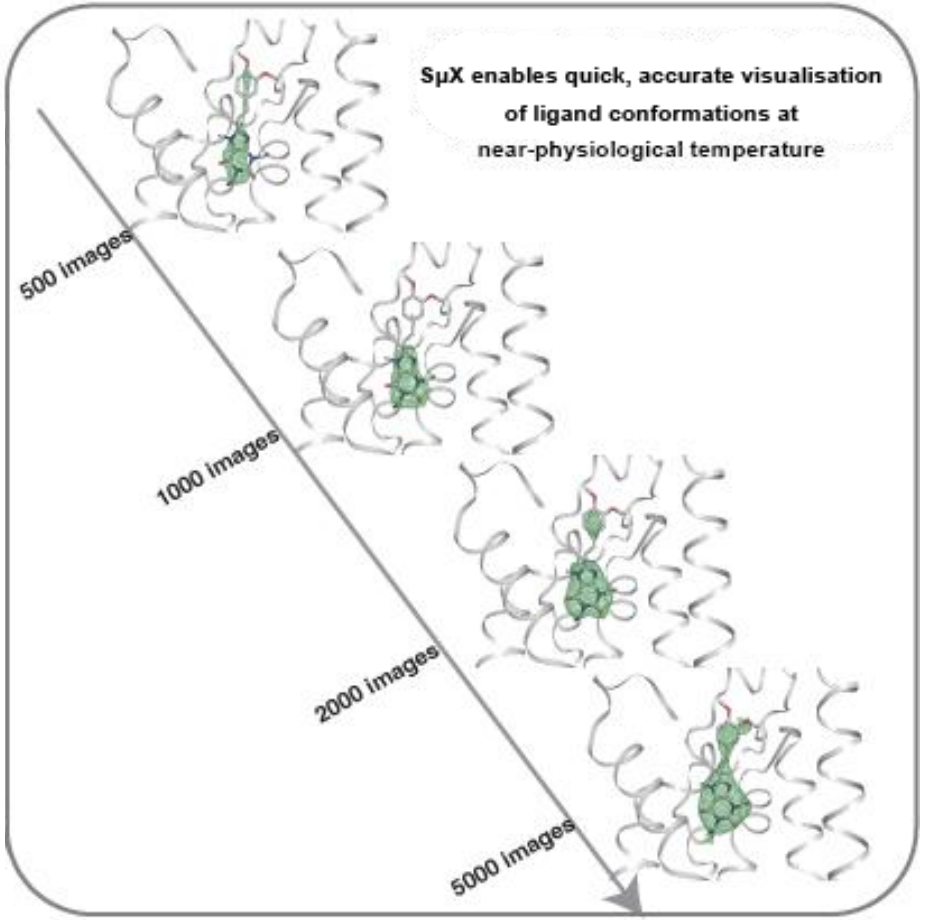
- We need to understand the structures of macromolecules under more **natural and realistic conditions**

- Not only observe the static structure of the protein but also analyse its function by tracking the **movement of its atoms**

EBSL8 – ID29: SERIAL CRYSTALLOGRAPHY (SµX)

in microsecond time scale



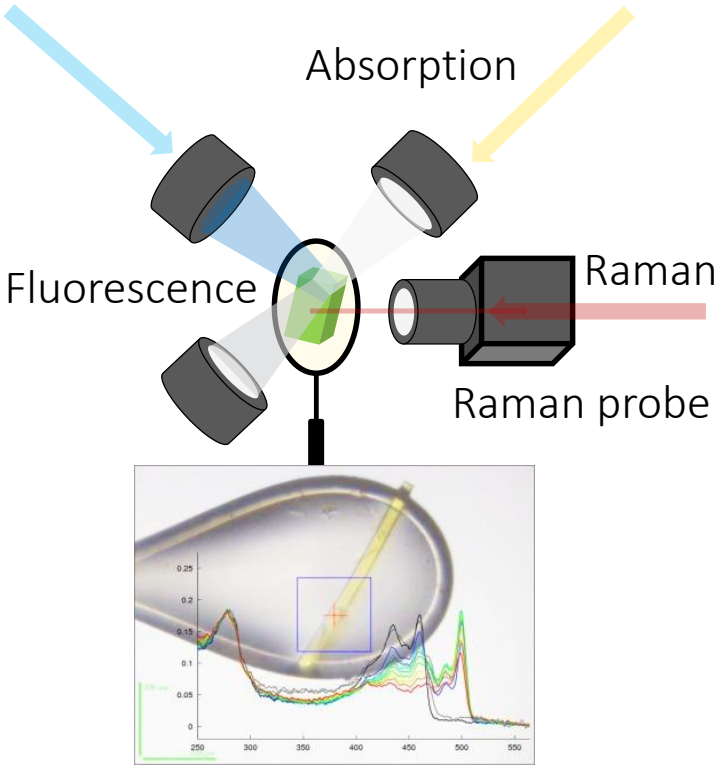


Orlans et al. Advancing macromolecular structure determination with microsecond X-ray pulses at a 4th generation synchrotron. Commun Chem. 2025. 8:6

BUILDING TOMORROW'S TOOLS FOR STRUCTURAL DISCOVERY: ADDING THE 4TH DIMENSION TO THE STRUCTURAL ANALYSIS

In crystallo spectroscopy (iCOS)

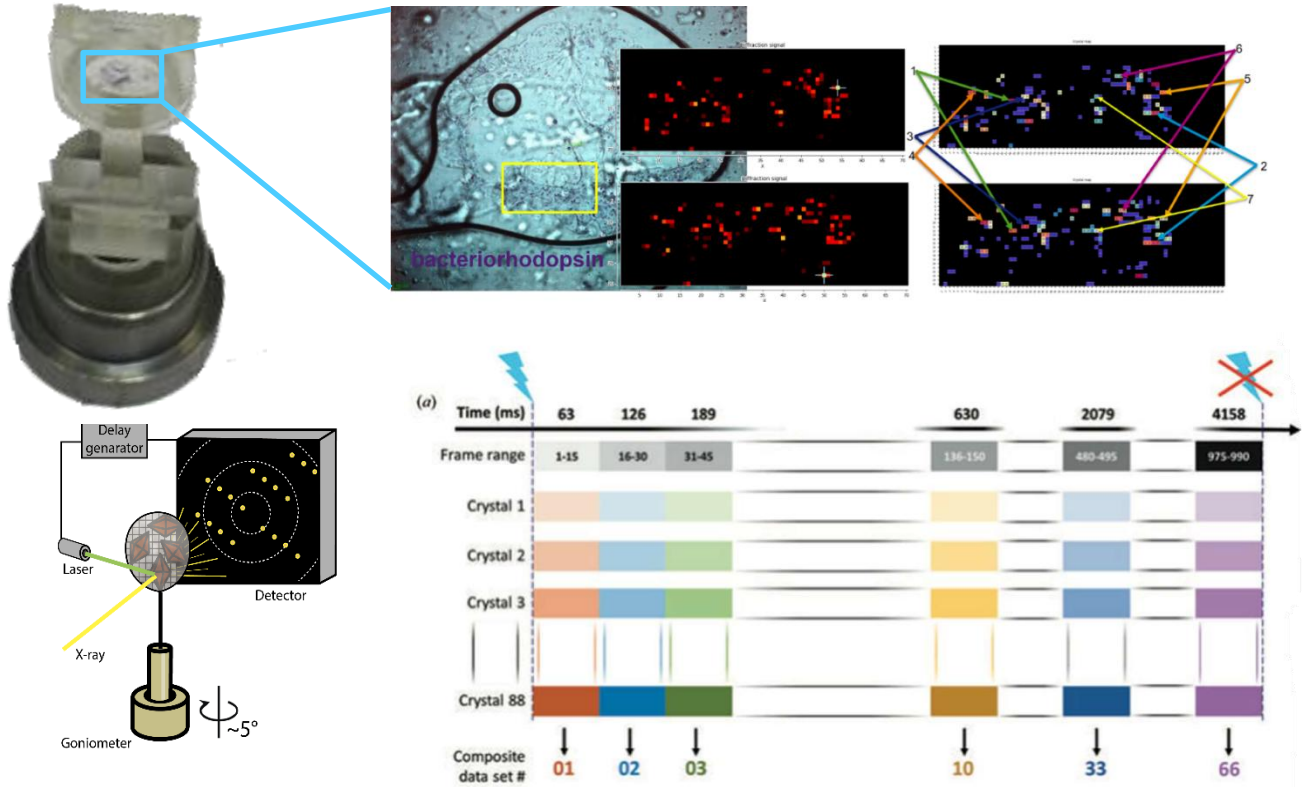
To probe protein dynamics from microsecond to hours



Engilberge et al. The TR-icOS setup at the ESRF: time-resolved microsecond UV-Vis absorption spectroscopy on protein crystals. Acta Crystallogr D Struct Biol 2024 80(Pt 1):16-25

ID30A3: time-resolved serial oscillation crystallography (TR-SOX)

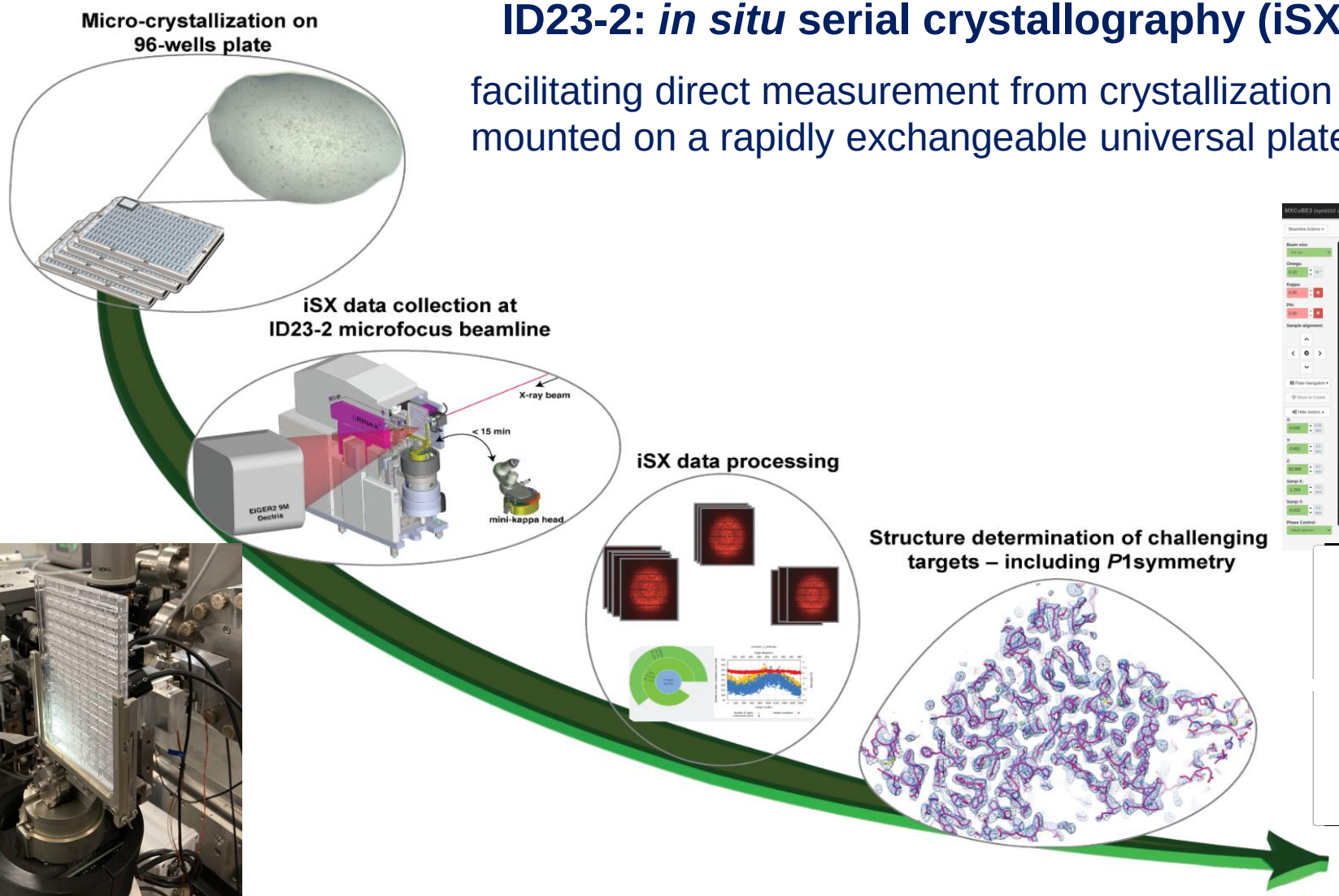
fast collection rates of small-sized crystals in the millisecond time-resolved range

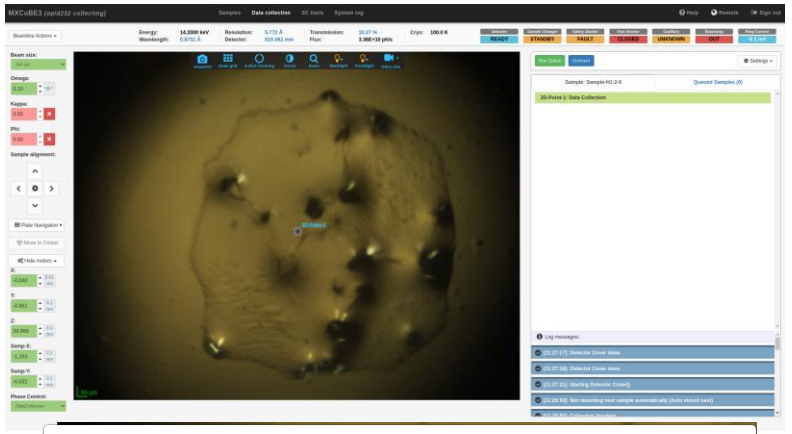


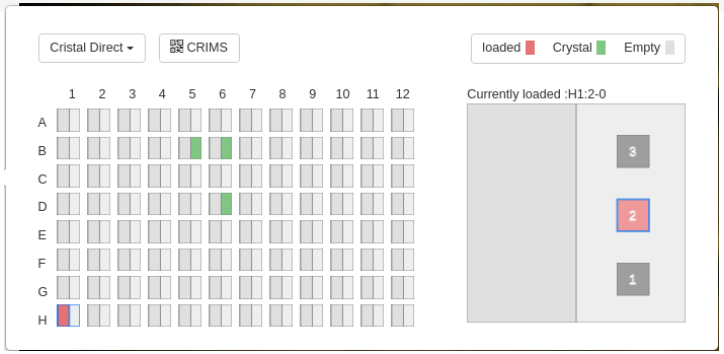
Aumonier et al. Slow protein dynamics probed by time-resolved oscillation crystallography at room temperature. IUCrJ 2022. 9(Pt 6):756

ID23-2: *in situ* serial crystallography (iSX)

facilitating direct measurement from crystallization plates mounted on a rapidly exchangeable universal plate holder



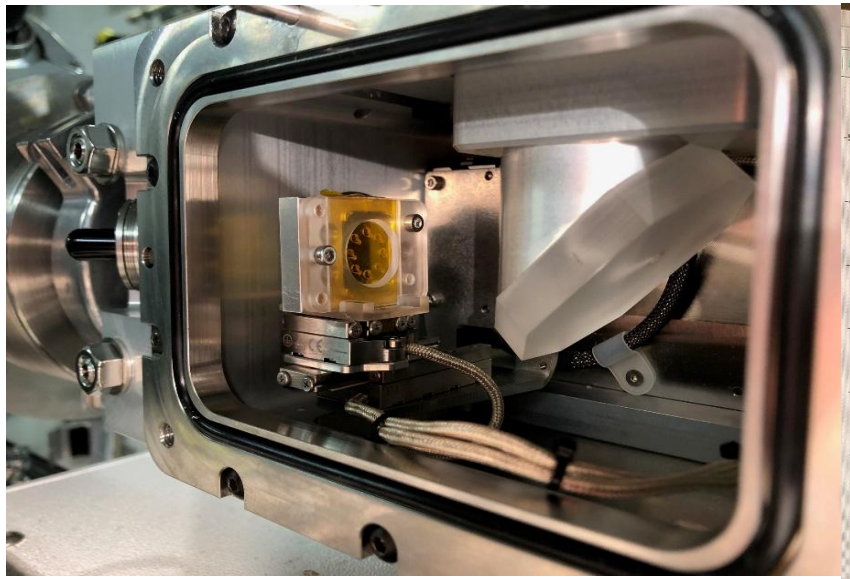




Foos N ,et al. In situ serial crystallography facilitates 96-well plate structural analysis at low symmetry. IUCrJ. 2024 Sep 1;11(Pt5):780

BM29: dynamics in solution using small angle X-ray scattering (bioSAXS)

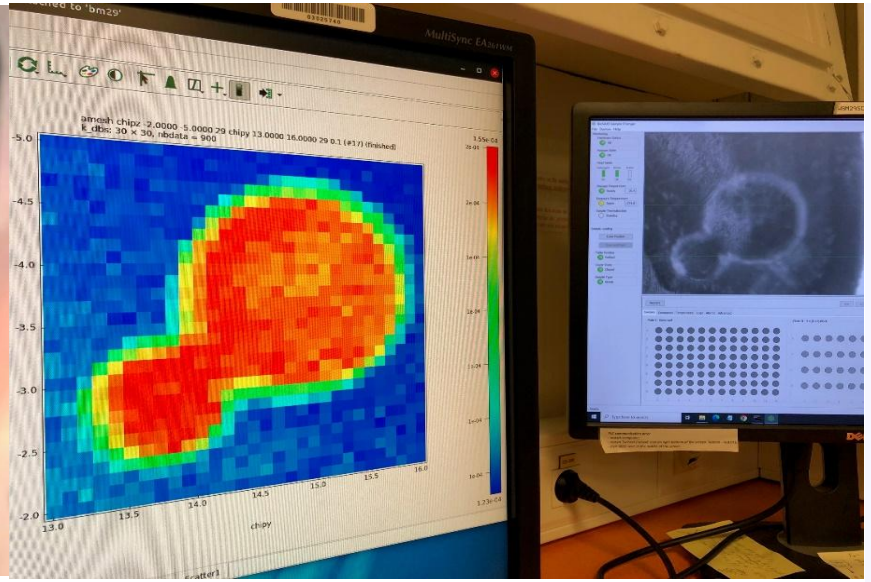
Sample Exposure Unit



Microfluidic chips (3D printed)

- Sample support designed + validated
- Microfluidic devices for gels
- Microfluidic device for flow mixing

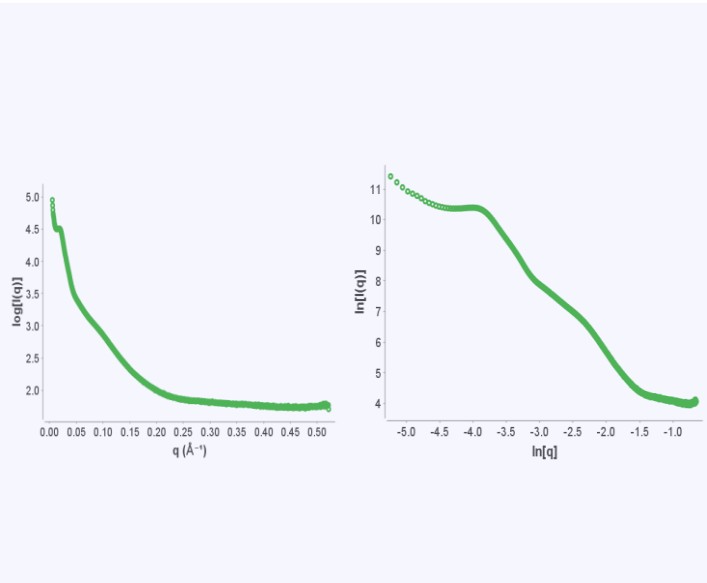
Data collection



Data collection possible

- Integration into BSxCuBE underway
- New data collection capabilities
- Upgrade to new pumps

Room temperature (RT)



Preliminary experiments

- Gels
- TR-SAXS

Courtesy of A. Popov (EMBL)

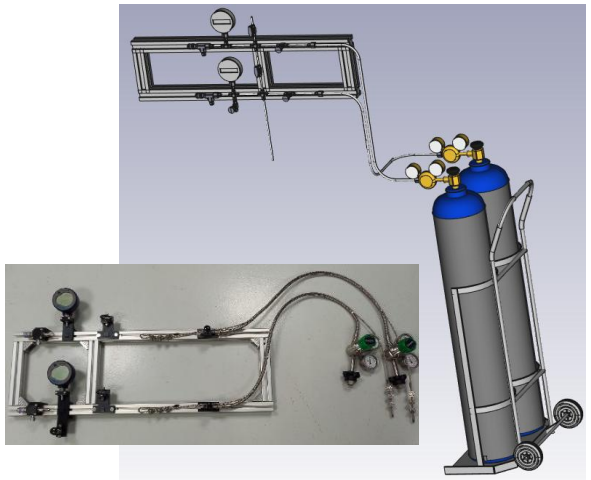
High-pressure MX platform (HPMX)

to investigate interactions between macromolecules and gases *in crystallo*

High pressure cells available

HP cell gas	He	Ar	Kr	Xe	O2	CO2	CH4
Pressure (bar)	2000	2000	50/500	30 (a)	70	50	50 (b)

(a) Xenon is currently unused due Xe gas-shortage and strong price increase
(b) The CH4 pressure cell is currently in in the manufacturing phase



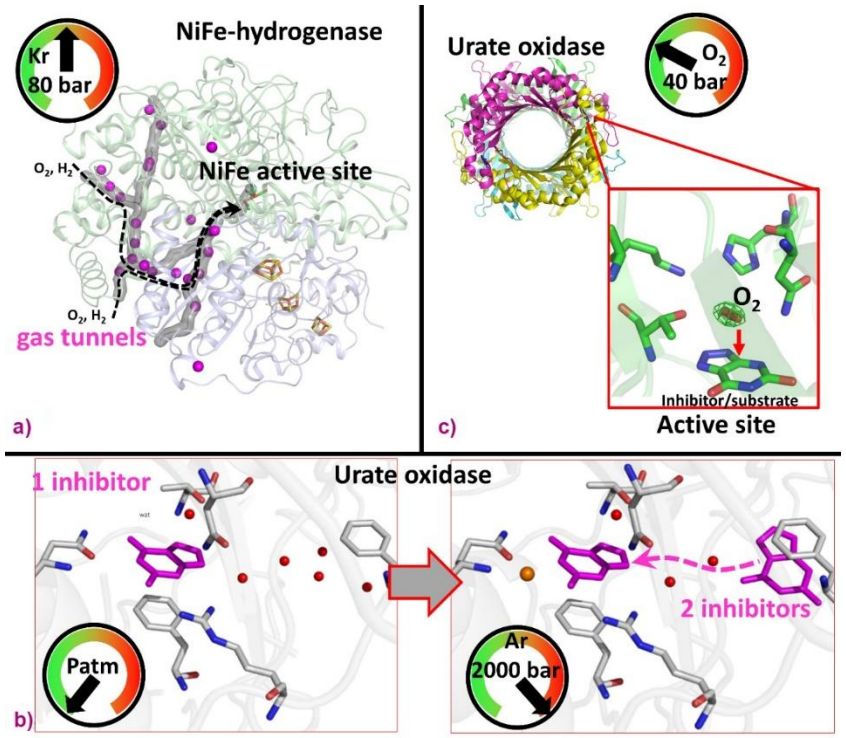
CO_2 pressure cell available at HPMX

APPLICATIONS

Elucidation of protein structural determinants using pressurised gases.

- Mapping of hydrogen diffusion tunnel using krypton
- Analysis of inhibitor binding conformations using argon
- Oxygen binding in active site by pressurised O_2

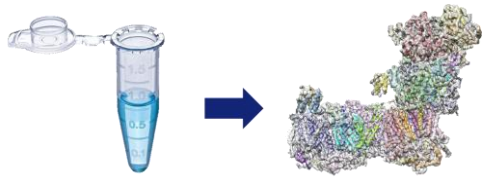
Courtesy of P. Carpentier (CEA)



Kalms et al., Angew. Chem. Int. Ed. 55, 5586-5590 (2016).
Prangé et al., Acta Cryst. D78, 162-173 (2022).
Lafumat et al., J. Appl. Cryst. 49, 1478-1487 (2016).
Melnikov et al., Commun. Biol. 5(1), 360 (2022).

BUILDING TOMORROW'S TOOLS FOR STRUCTURAL DISCOVERY: CRYO-EM INTEGRATION INTO THE MACROMOLECULAR ARCHITECTURE PIPELINE

Solution-tO-Structure pipeline (SOS)

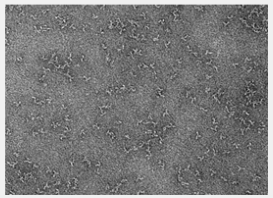


Entry point 1
Starting from liquid sample

Entry point 2
Starting from vitrified unscreened grids

1. Negative Stain EM
2. Vitrification
3. Cryo-EM grid screening
4. Image processing
5. Krios data collection

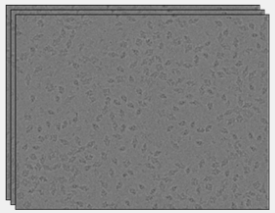
- Verification of sample quality by Negative Stain EM using different stains – done once



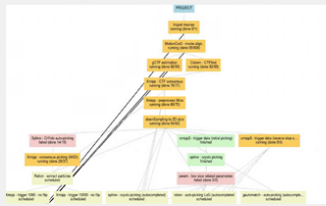
- Sample vitrification with varying grid types and sample concentrations.

3X

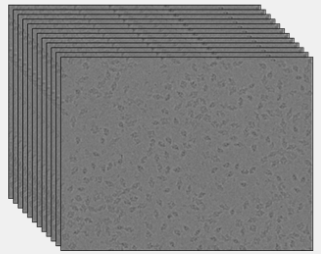
- Grid screening and subsequent small data collection in Glacios 200kV microscope.



- Data processing to obtain preliminary characterization results, showing high-resolution potential



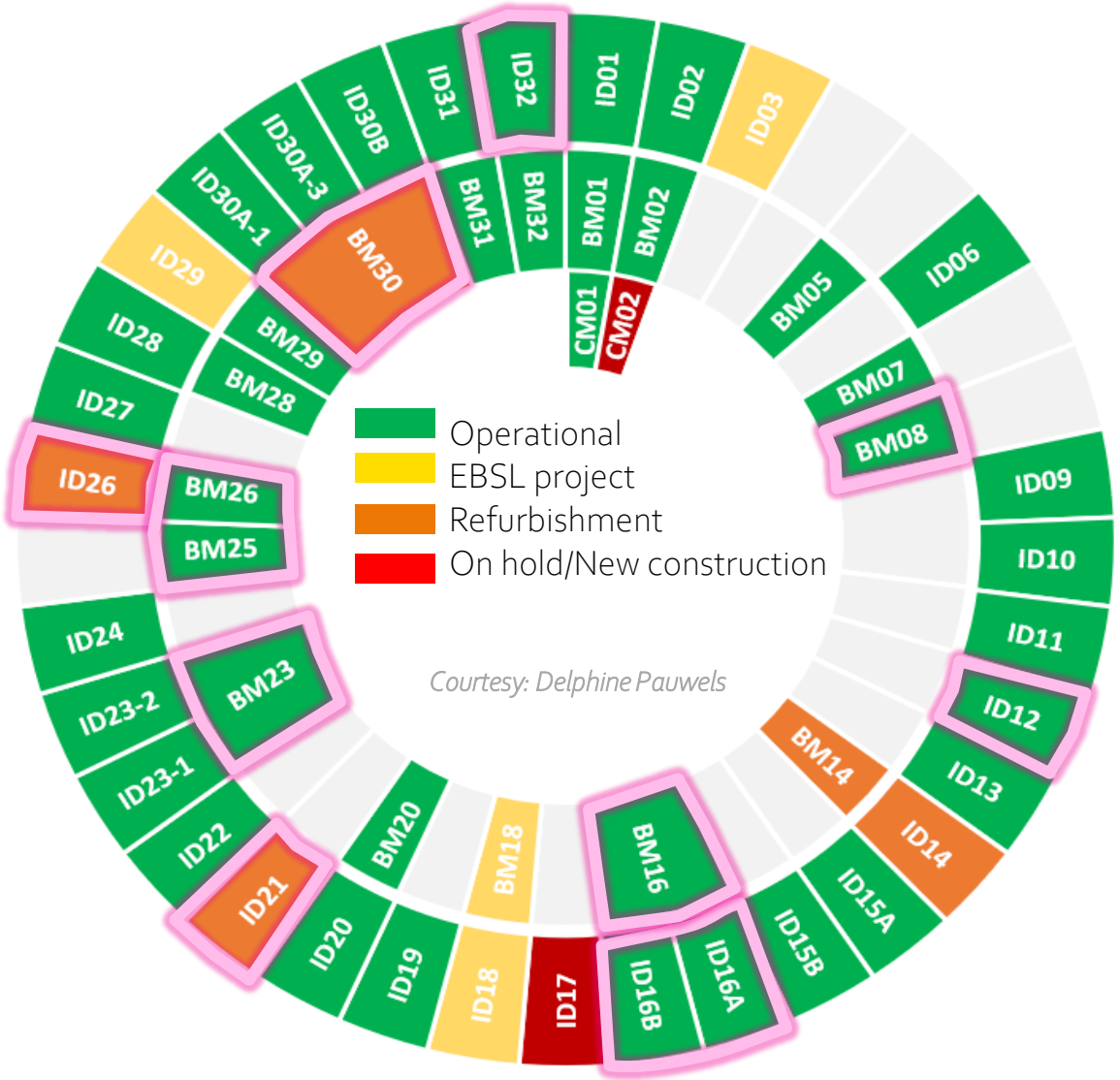
- High-resolution larger data collection in CM01 Krios microscope



Courtesy of E. Kandiah

ESRF BEAMLINE PORTFOLIO FOR BIOLOGY RESEARCH AT CELLULAR AND SUBCELLULAR RESOLUTION

Synchrotron spectroscopy is highly relevant to provide detailed **chemical information** of biological molecules in their native environment and in a dynamic process

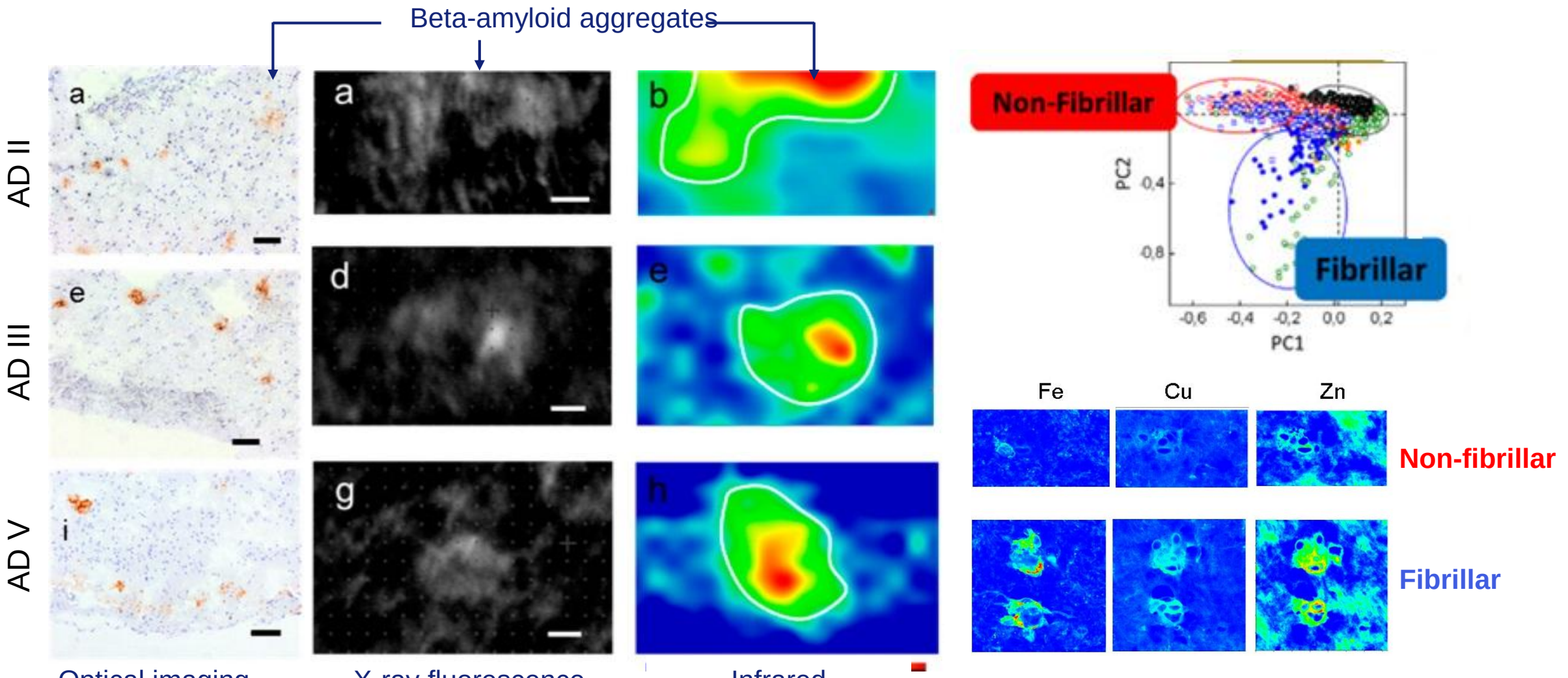


X-ray Absorption Spectroscopy (XAS) measures the absorption of X-rays by atoms in a sample. It provides information about the electronic and structural properties of materials, including oxidation state, coordination environment, and local atomic structure.

Infrared Microspectroscopy (IR) Utilizes infrared light to analyze the chemical composition and molecular structure of biological samples, offering insights into biomolecular interactions and cellular processes.

X-ray Fluorescence (XRF) microscopy maps the distribution of elements within biological samples. It can provide insights into elemental composition and distribution within cells, tissues, and organs.

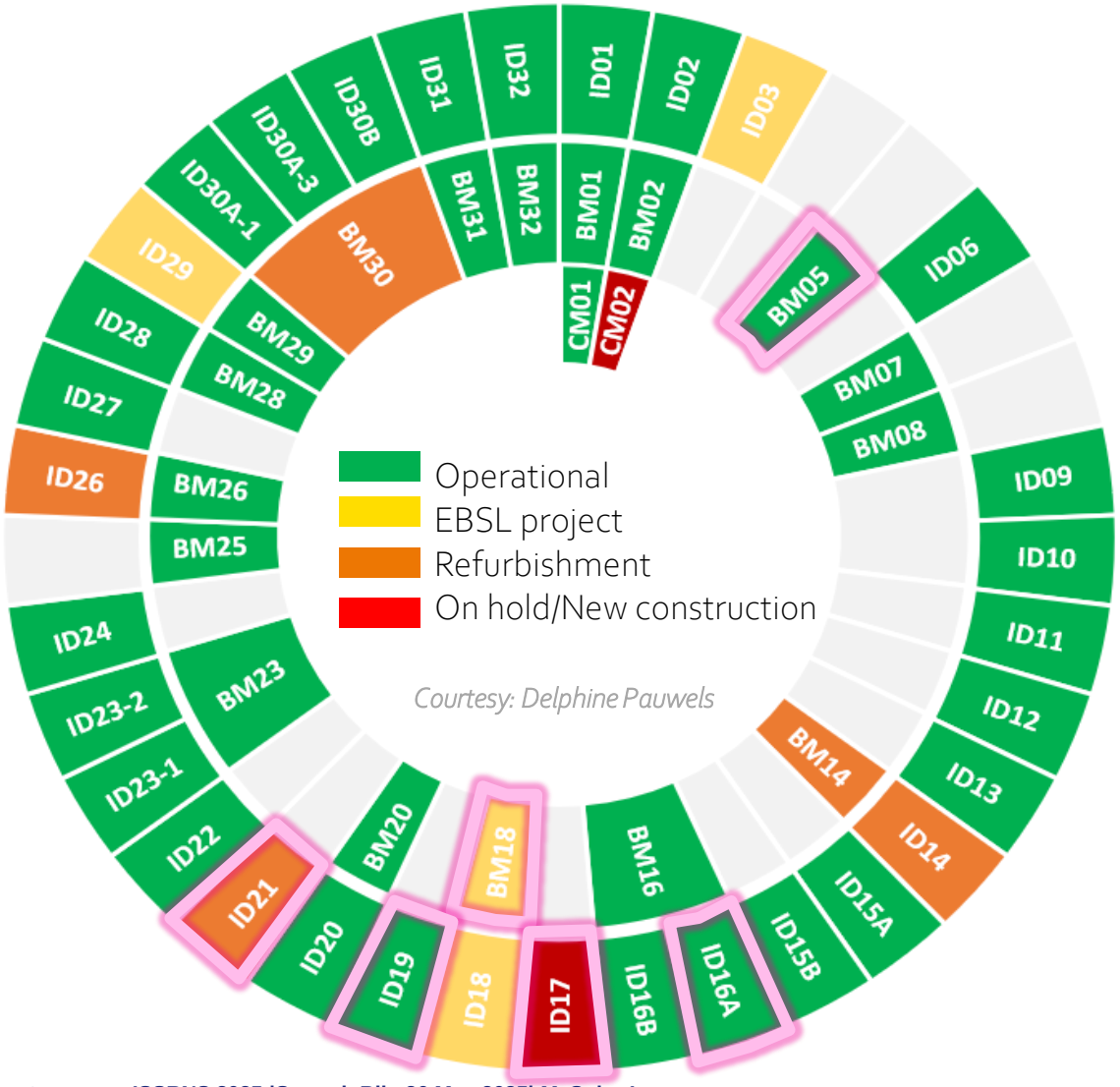
ID16B, ID21: SPECTROSCOPY TO ANALYSE DISEASE PROGRESSION AT THE (SUB-)CELULAR LEVEL



Álvarez-Marimon et al. ACS Chem. Neurosci. 2021

ESRF BEAMLINE PORTFOLIO FOR BIOLOGY RESEARCH AT CELL / TISSUE / ORGAN LEVELS

Synchrotron bioimaging offers precise, **non-invasive examination of organs and organisms**, revealing intricate internal structures and processes across scales, facilitating advanced research in biology and medical sciences.



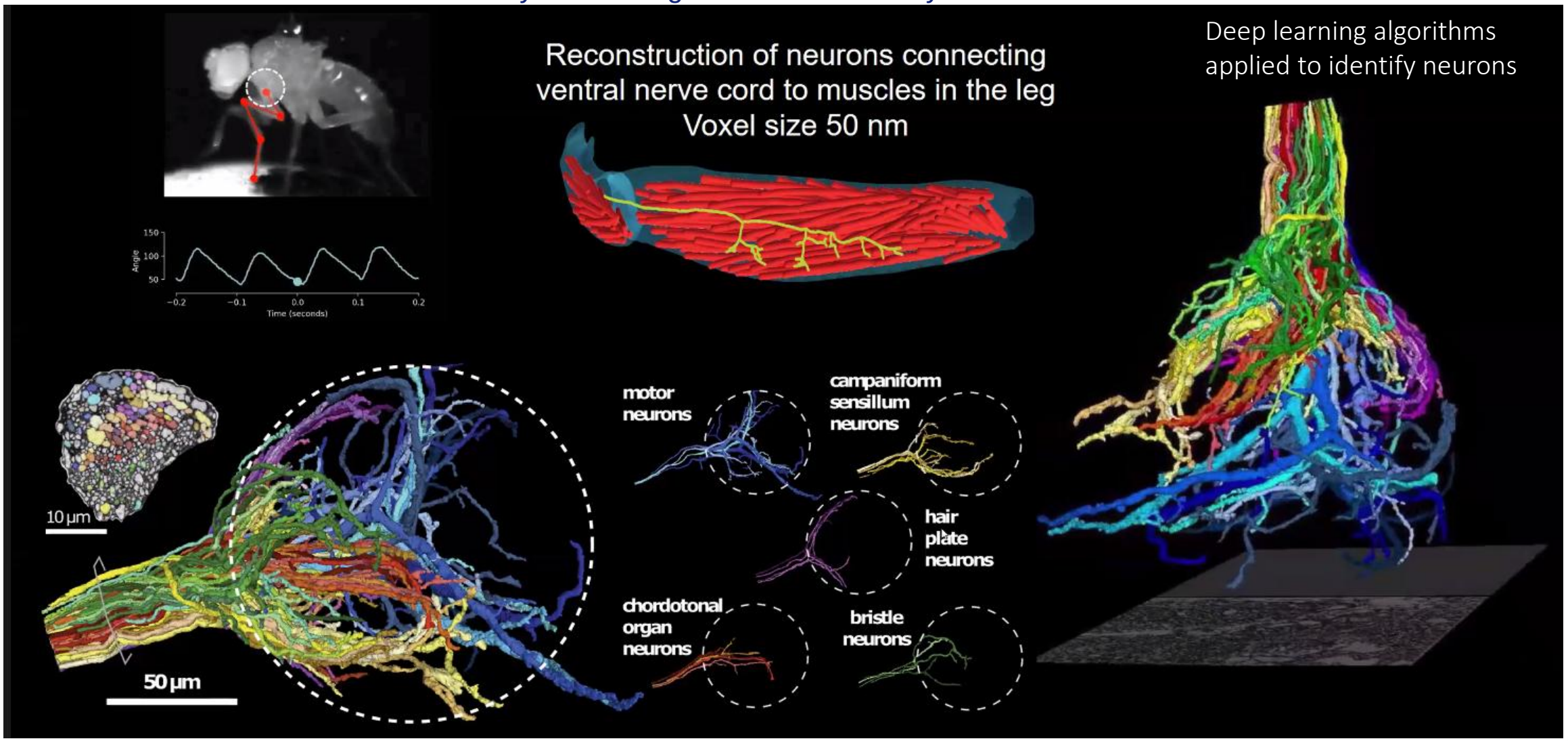
X-ray Microscopy (XRM) imaging of cellular and subcellular structures without the need for staining or sectioning. It allows for the visualization of organelles, cell membranes, and other cellular components.

X-ray Tomography is similar to XRM, but with the capability to reconstruct three-dimensional images of thicker samples or tissues.

X-ray Computed Tomography (CT) scans provide detailed cross-sectional images of tissues and organs within the body. It is widely used in medical diagnostics and research to visualize anatomical structures and detect abnormalities.

ID16A: NANO-IMAGING BEAMLINE FOR NEURONAL RECONSTRUCTION

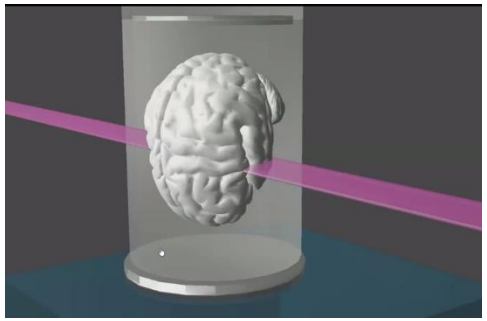
reconstruction of dense neural circuits in 3D, comprehensively cataloging neurons and even tracing individual neurons from muscles to the central nervous system in large volumes of fruit fly nervous tissue



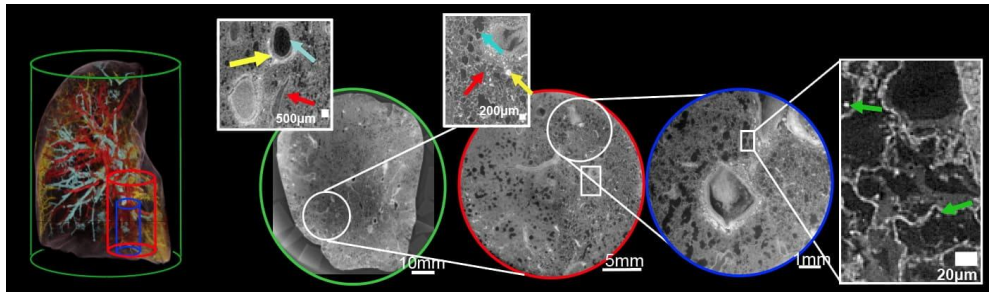
Kuan et al. Nature Neuroscience 2020; Winding et al. Science 2023

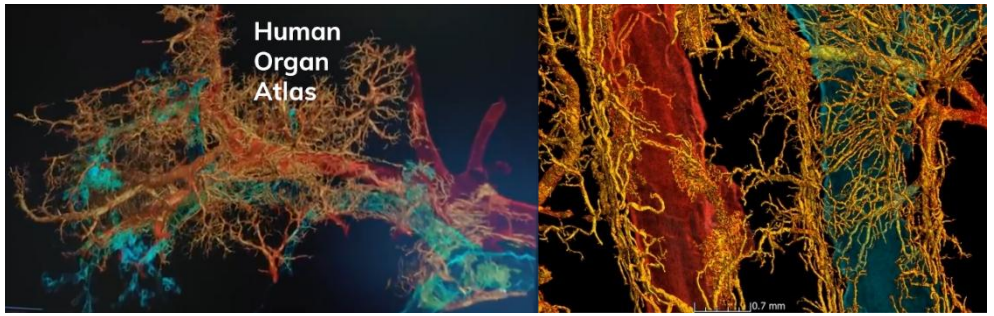
Courtesy A. Pacureanu

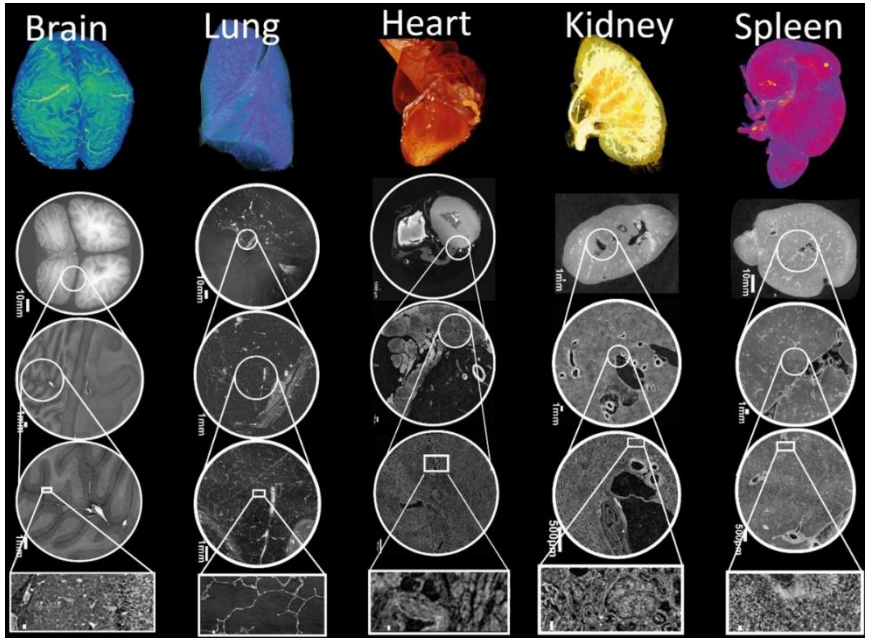
BM18: ZOOMING IN AND OUT WITHIN HUMAN ORGANS AT MULTIPLE LENGTHS SCALES



Walsh et al. Nature Methods 18, 1532 (2021)



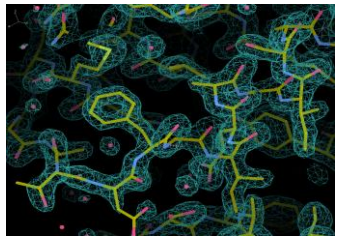




The Human Organ Atlas https://human-organ-atlas.esrf.fr/
10 donors, 24 organs, 178 datasets available

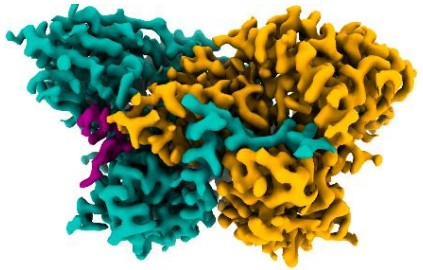
Courtesy of P. Tafforeau / G. Martínez-Criado

LOOKING AHEAD: ESRF-EBS FOR INTEGRATIVE BIOLOGY RESEARCH ACROSS SCALES



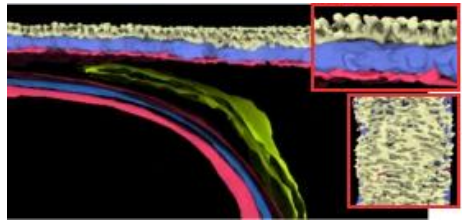
Macromolecular Crystallography
0.7 – 3 Å

ID23-1, ID23-2, ID29,
ID30A-1, ID30A-3
ID30B, BM07, ID13



Cryo-electron Microscopy
1.5 – 15 Å

CM01, CM02

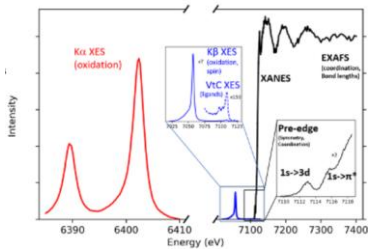


Cryo-electron Tomography
20 – 50 Å

CM01, CM02

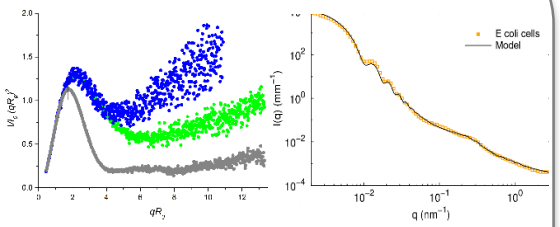
X-ray Nanotomography
10 – 100 nm

ID16A, ID16B, ID18



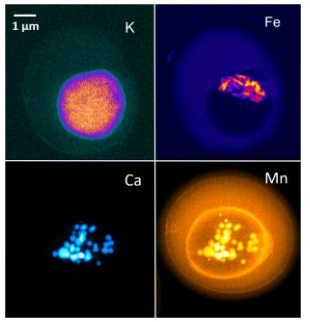
X-ray Spectroscopy
< 1 Å

ID12, ID24, ID26
ID32, BM23
BM26A, BM30



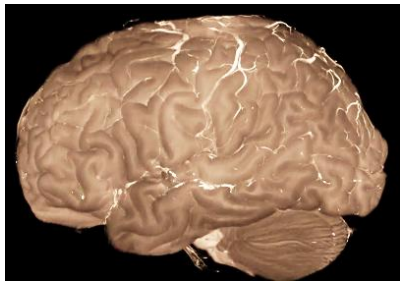
Small-angle X-ray scattering
0.5 nm – 5 µm

BM29, ID02
ID09, ID13, BM26B



X-ray Fluorescence
20 nm – 1µm

ID16A, ID16B, ID21



X-ray Microtomography
0.8 – 50 µm

ID19, BM05, BM18