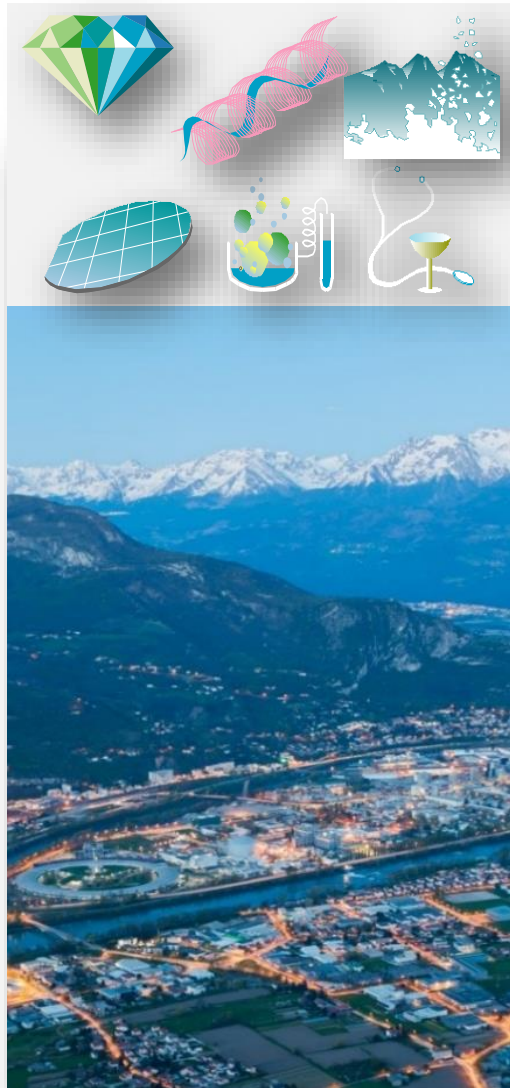


Structural Biology at the ESRF – opening up new avenues

Montse Soler-Lopez
Head of Structural Biology Group





- Beamlines/Laboratories in the structural biology group
- Structural Biology at the ESRF Today
- New avenues:
 - Adding the 4th dimension to the structural analysis
 - The use of pressurized gases in structural biology
 - Drug discovery with fully automated beamlines
 - From solution to EM structure: SOS pipeline
 - Beyond single particle analysis, cryo-Electron Tomography

X-ray Macromolecular crystallography (MX)

With  EMBL
European Molecular Biology Laboratory

- **ID30A-1:** fully automated 12.8 keV, 20-100 μm
- **ID30B:** } tunable 6-20 keV (2.0-0.62 \AA), 20-50 μm
- **ID23-1:** }
- **ID30A-3:** minifocus 12.9 keV (0.96 \AA), 15 μm
- **ID23-2:** microfocus 14.2 keV (0.87 \AA), 5 μm
- **EBSL8 (ID29):** serial, nanofocus 10-25 keV (1.24- 0.5 \AA), 0.5 μm

With  ibs
Institut de Biologie Structurale

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

Small angle X-ray scattering (SAXS)

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

Cryo-Electron Microscopy

With  ibs Institut de Biologie Structurale

EMBL  European Molecular Biology Laboratory

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

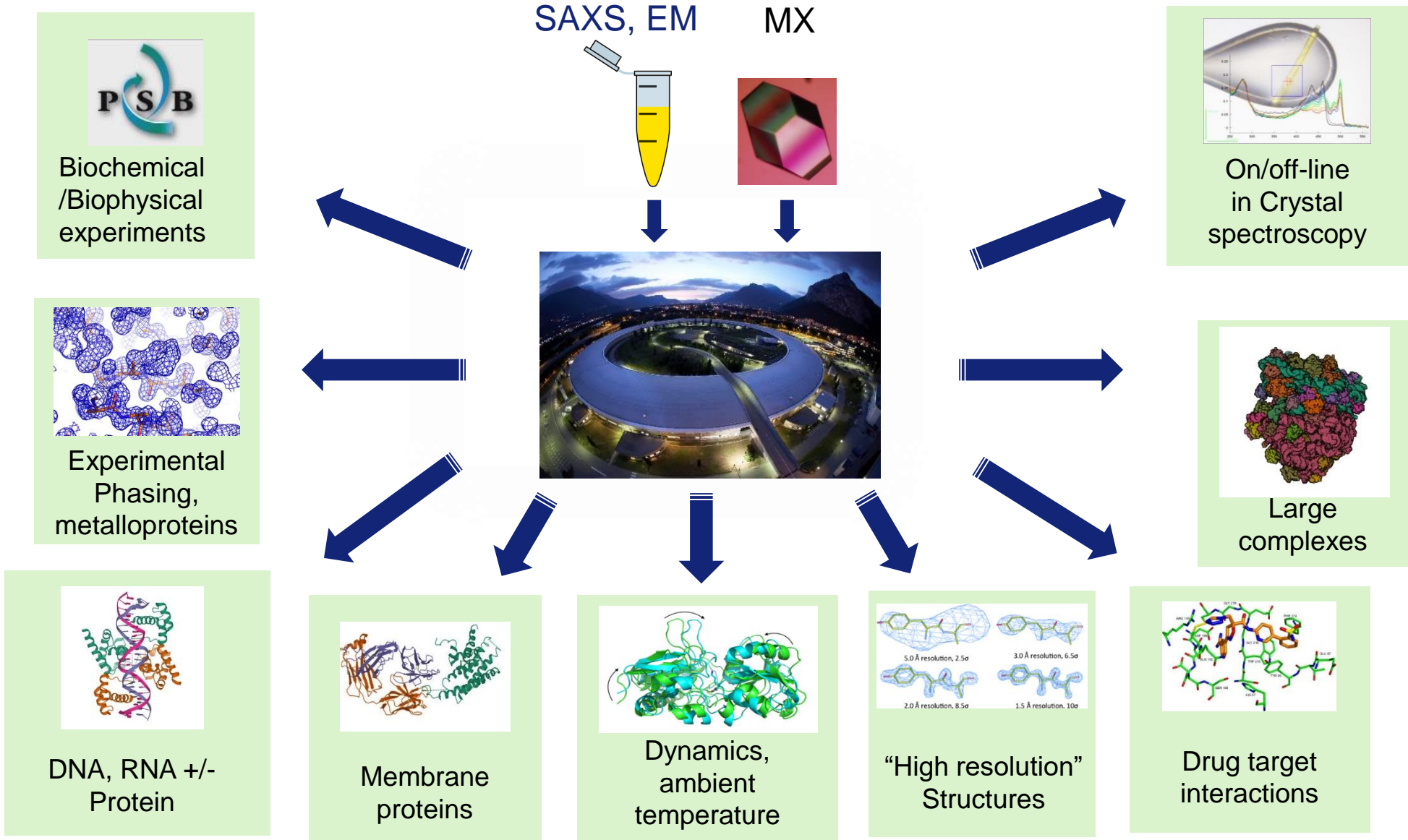
Complementary methods

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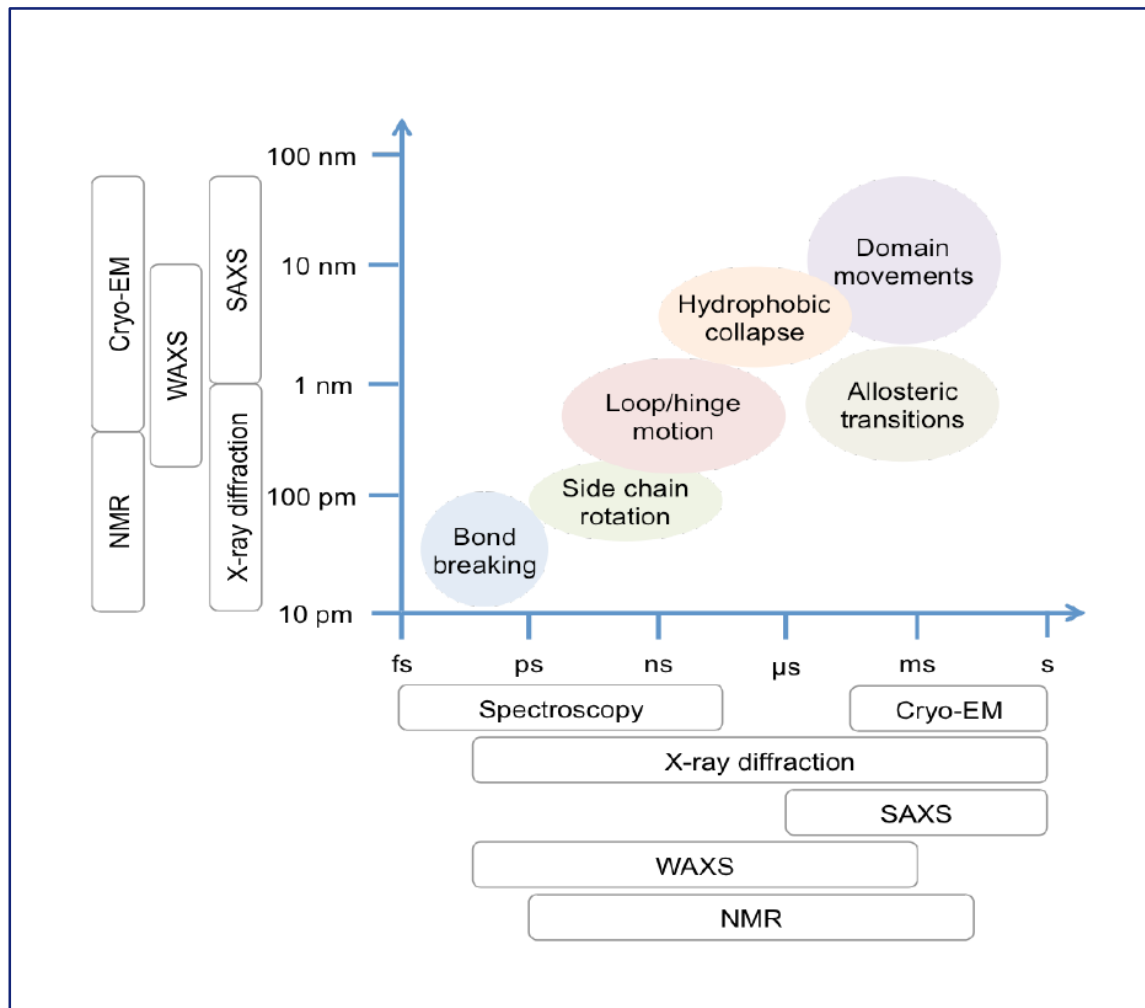
- **icOS:** *In crystallo* optical spectroscopy: UV/Vis absorption, fluorescence, Raman

With  cea
GRENOBLE

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



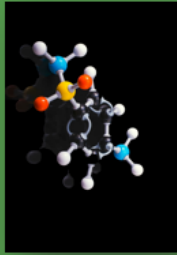
Time-resolved serial crystallography (TR/RT SSX)



- Repeat many times to build up signal to noise.
- Repeat with different values of Δt to build up a series of time-resolved datasets

- Millisecond or microsecond time resolution
- Caged compounds at RT; reactions initiated by laser or X-ray pulse (photo/radio-active substrate or prosthetic group).
- Requires *in crystallo* and *in solutio* spectroscopy
- Molecular movies

Time-resolved serial crystallography (TR/RT SSX)

Drug design

- Exploit room temperature fragment screening
- Identify time dependent structure: ligand complexes

Biofuel

- Characterize and optimize biochemical processes for production new carburants
- Exploit novel sources for bioenergies

Enzymology

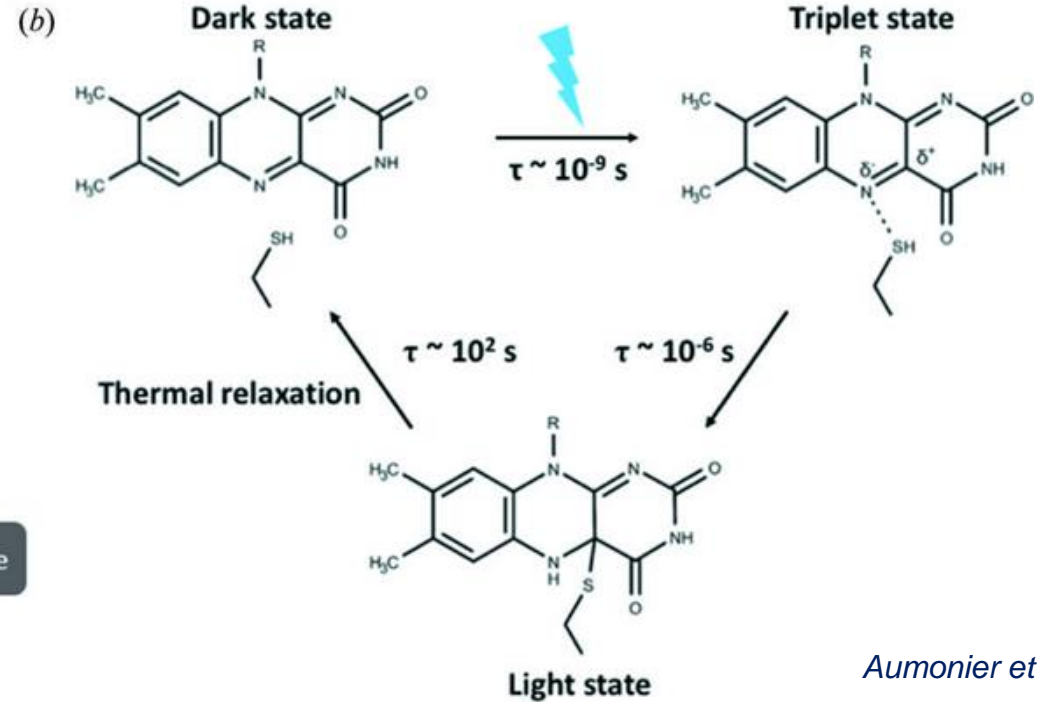
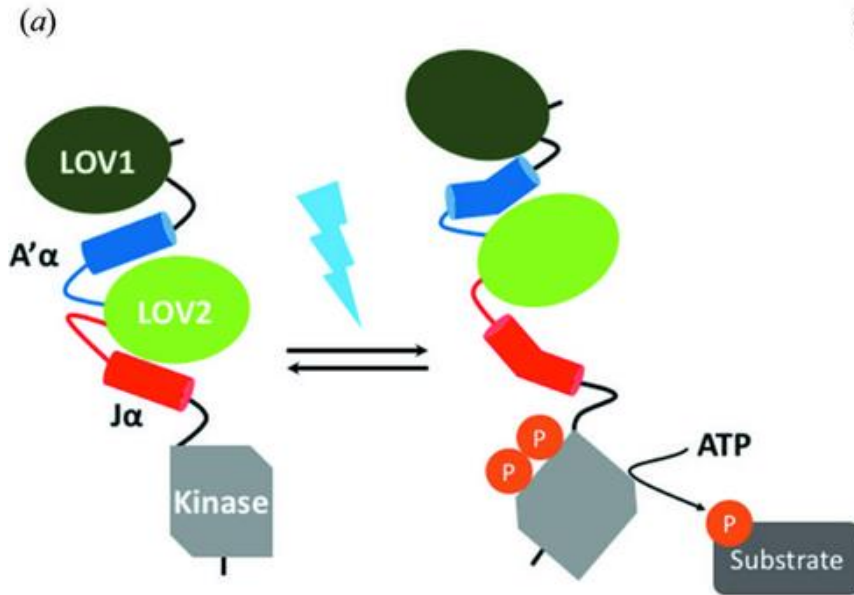
- Study enzymatic reaction in crystals
- Enzyme design and repurposing by synthetic biology

Serial and Time resolved Crystallography**Photobiology**

- Study light activatable biological processes
- Investigate light dependent biochemical reaction

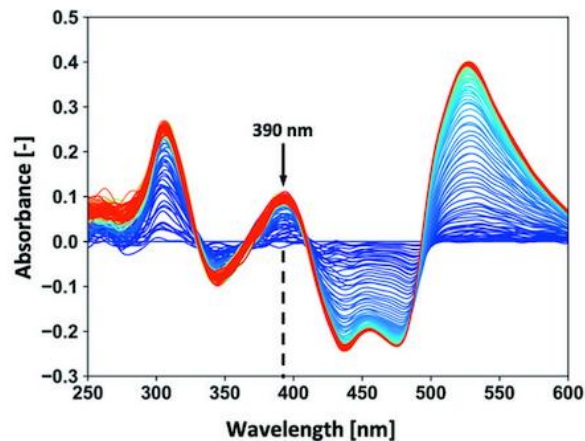
Bioremediation

- Study and engineering of macromolecular complexes involved in bioremediation
- Develop enzymatic processes for plastic waste treatment

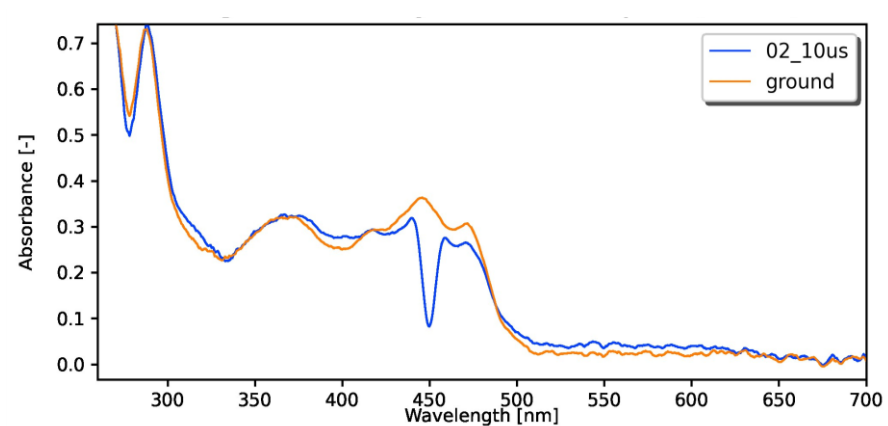


Online in crystallo Raman/UV-vis absorption

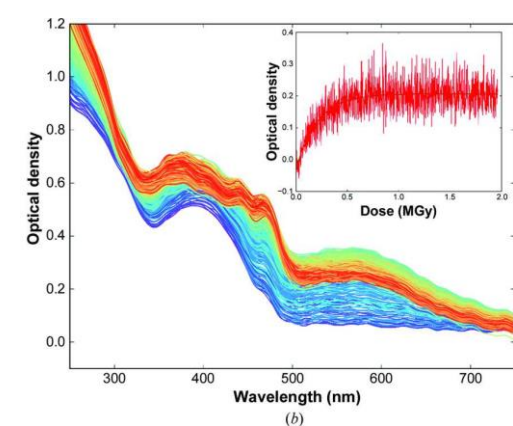
Aumonier et al. IUCr 2020



Millisecond to second timescale



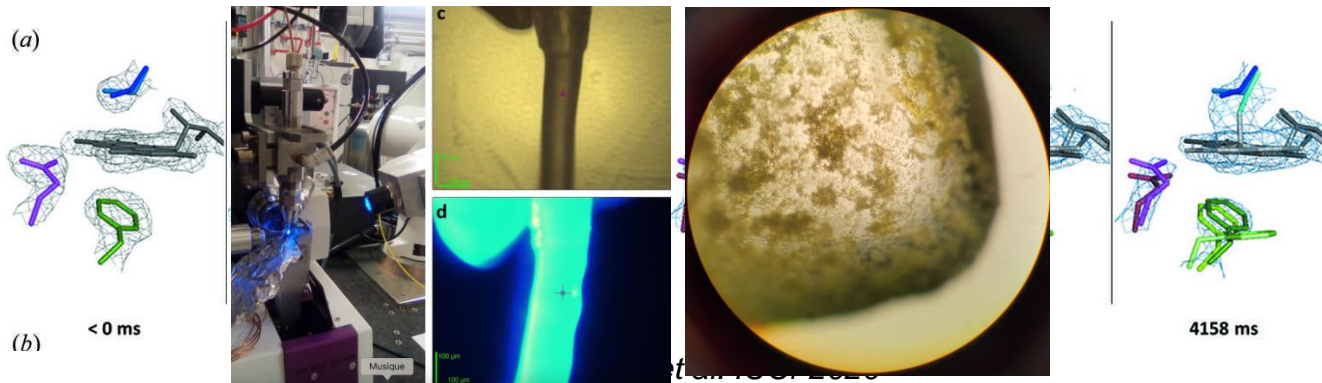
Microsecond to second timescale



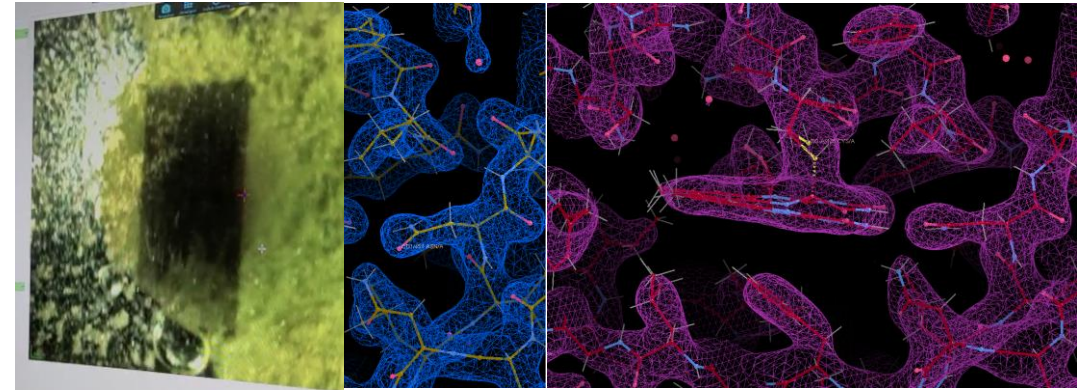
Radiation damage at cryo

Millisecond to second timescale

ID30A-3: RT-TR-SOX with jet

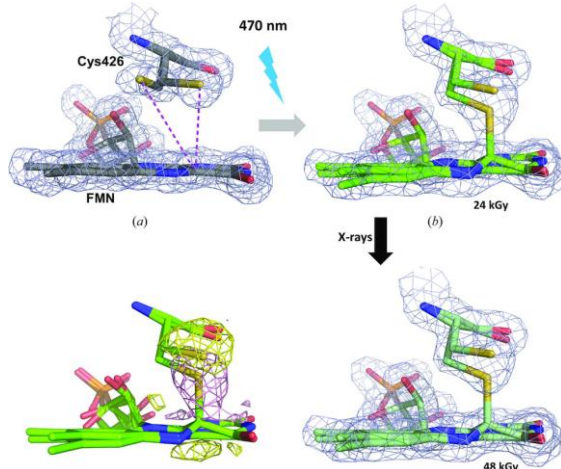


ID23-2: RT-TR on SOS chip



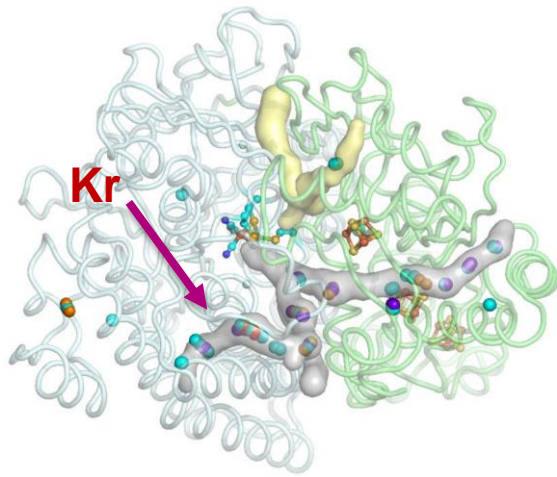
cryotrapping + radiation damage

ID30B: Raman



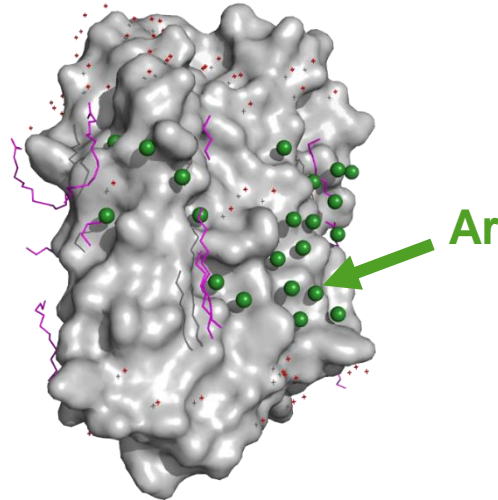
Microsecond timescale,
High resolution diffraction

ID29
Serial and time
resolved
crystallography



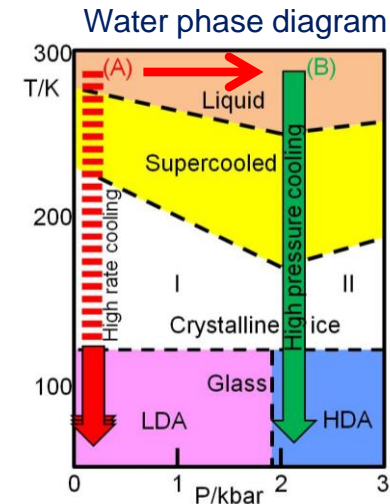
Mapping functional channels
in proteins

Zacarias, S. et al., JBIC, (2020)

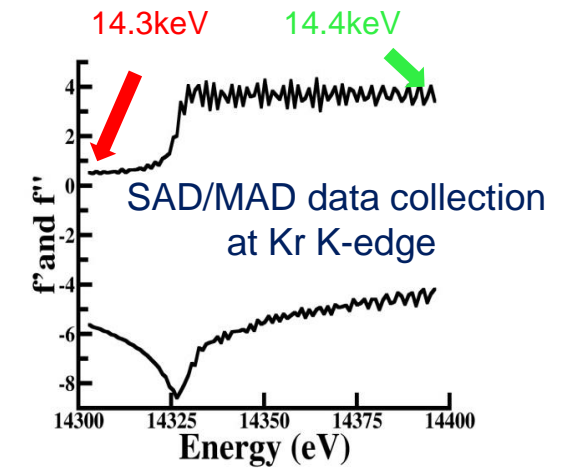


Probing surface functional cavities
of proteins (allosteric effect)

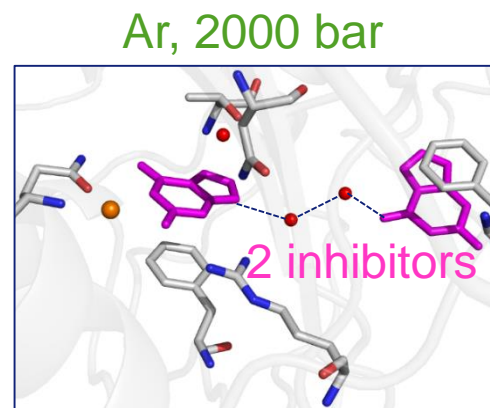
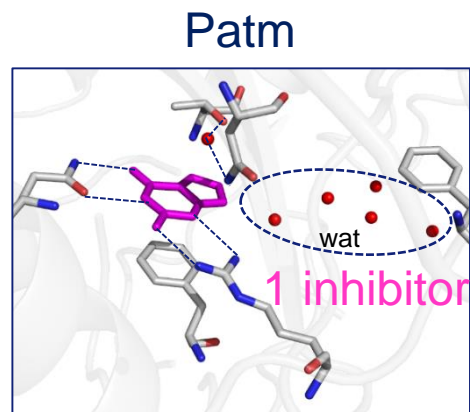
Melnikov, I. et al., Comm. Bio., (2022)



Crystals flash-cooling without cryo-protection.
Noble gases derivatives structure determination

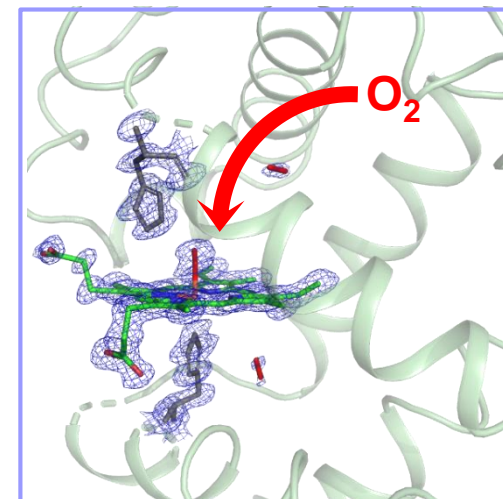


SAD/MAD data collection
at Kr K-edge

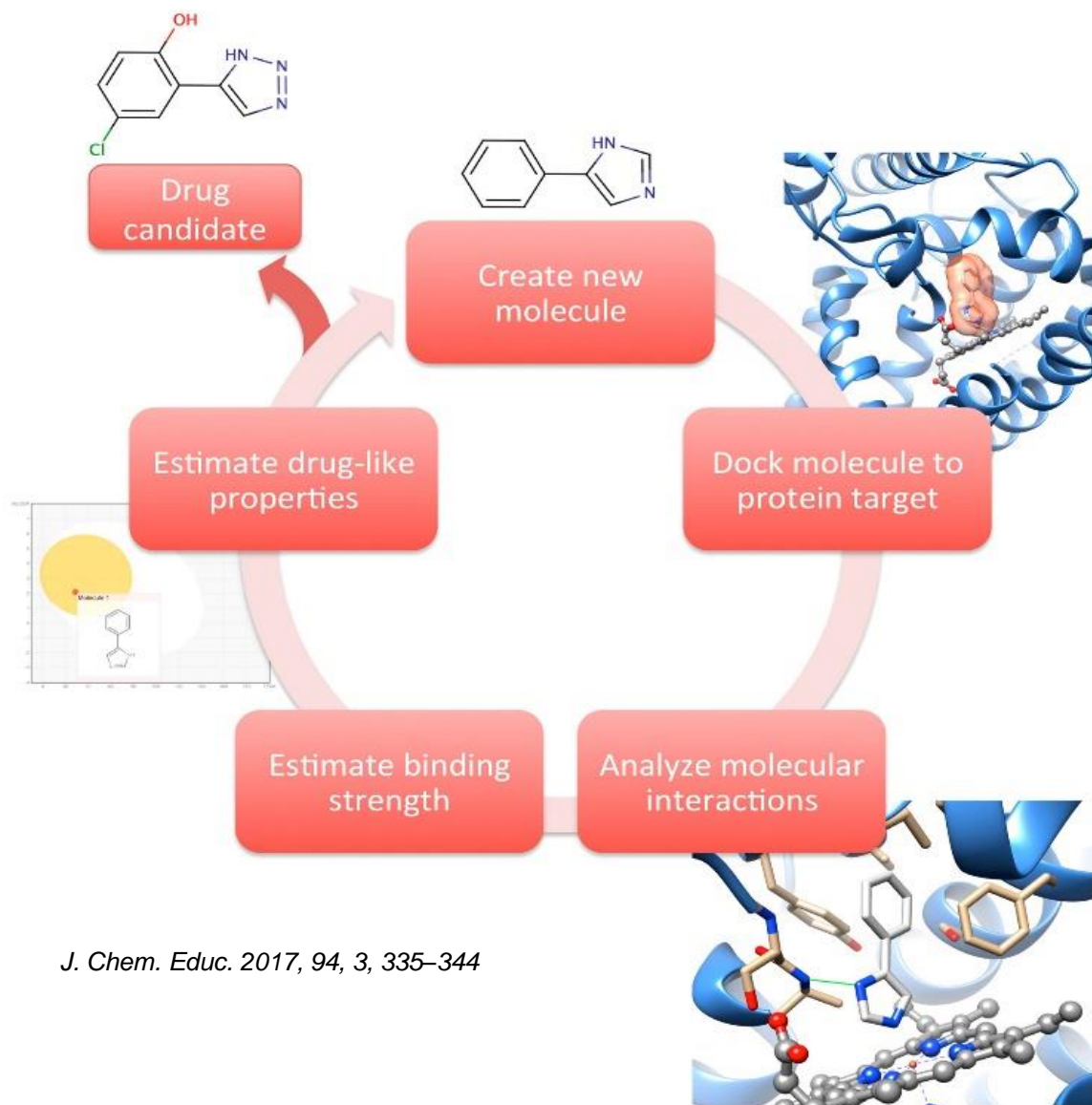


Pressure induced local structural modifications for ligand binding

Prangé, T. et al., Acta Cryst. D, (2022)



Mechanistic analysis of
binding sites in gas
dependent proteins
(O₂, CO₂)



MASSIF-1, ID30B

Before the experiment

on-line booking

User office invitation

Samples declaration

Samples shipping

Experiment design

During the experiment

Crystal mounting

Crystal centering

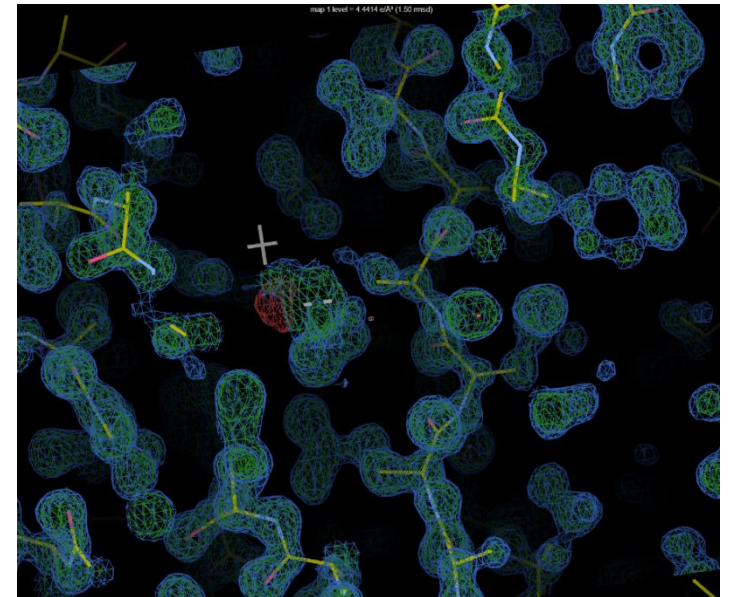
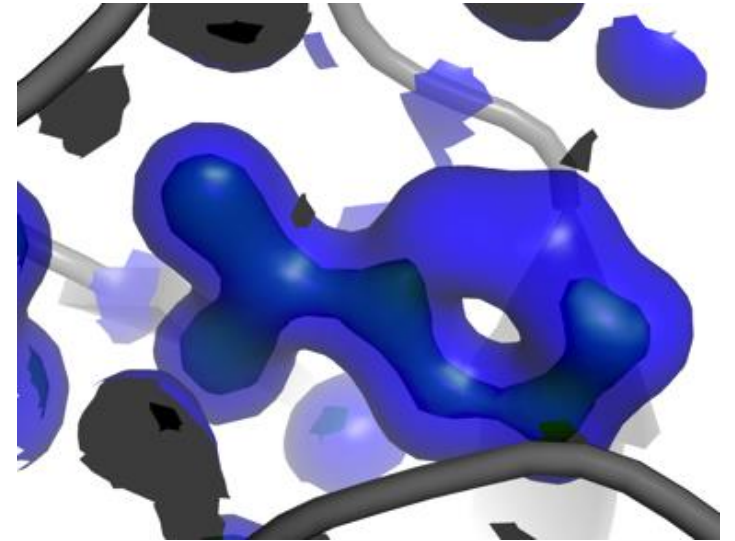
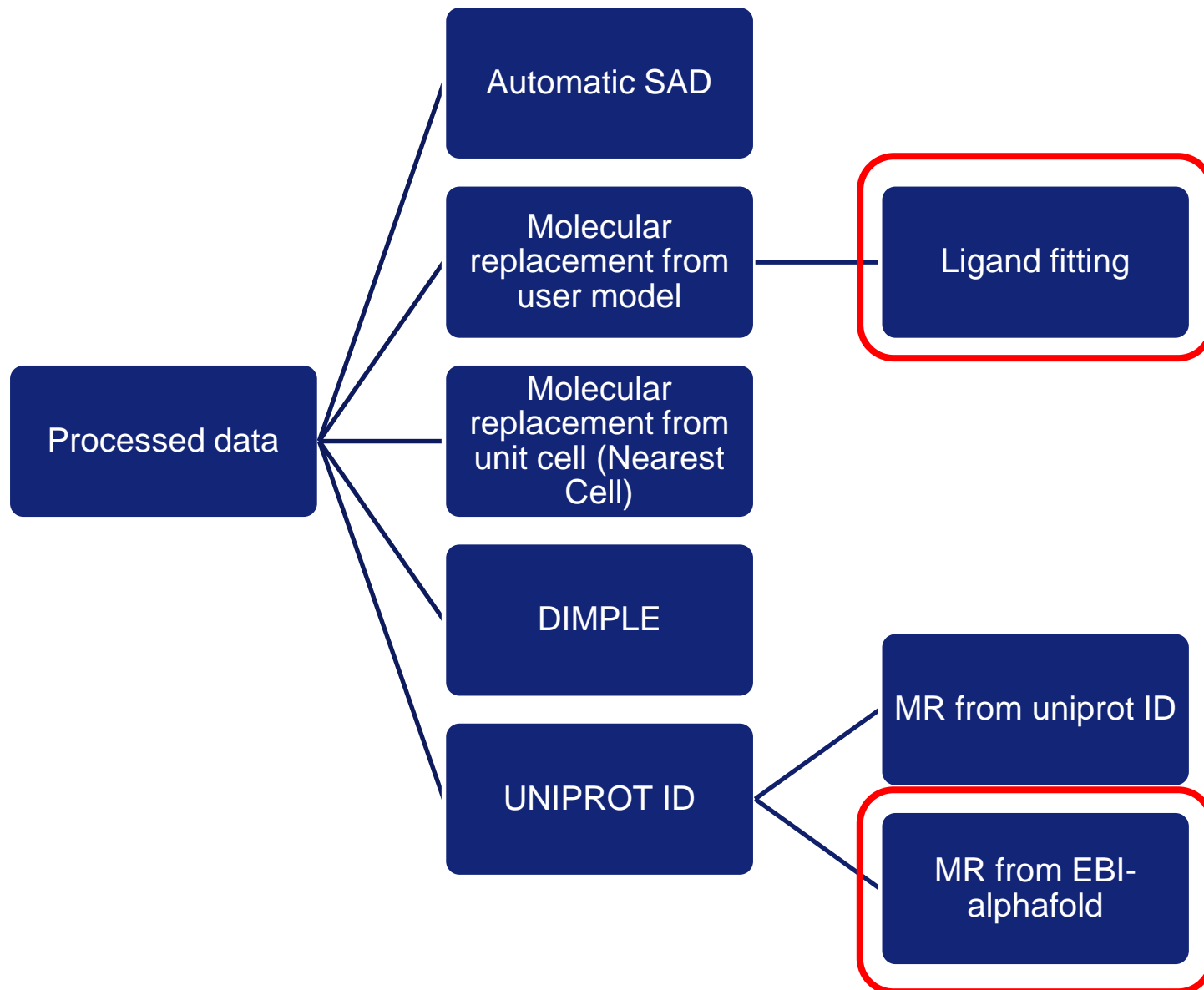
Diffraction plan

Data collection

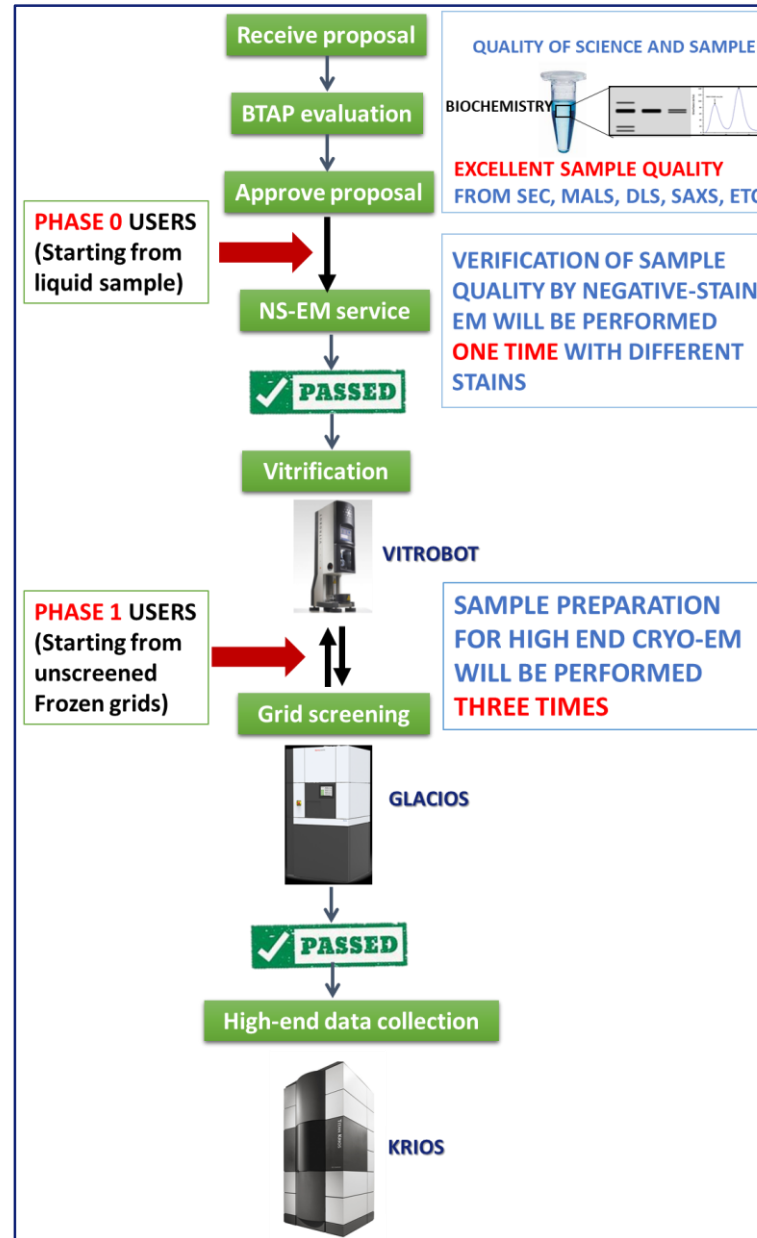
After the experiment

Data collection processing

Structure determination



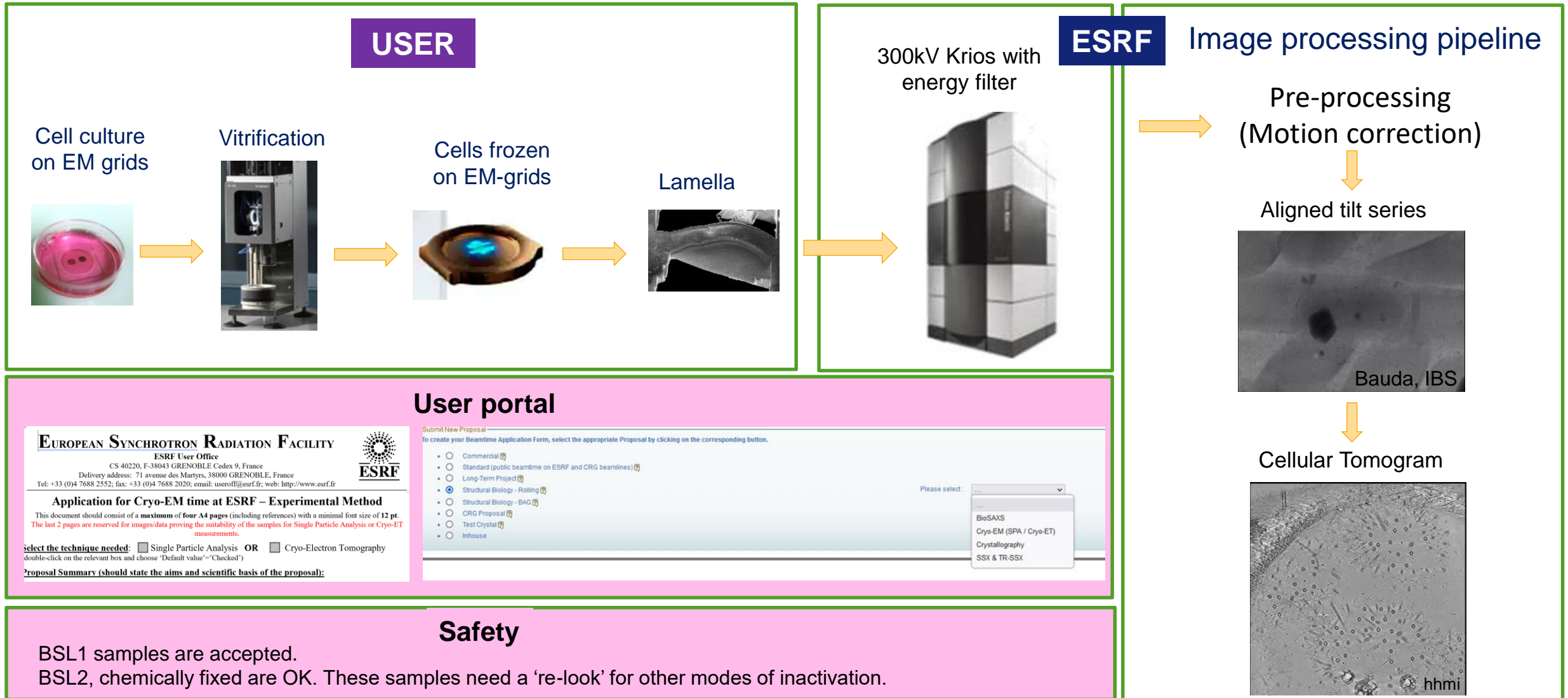
Courtesy of M. Nanao



www.esrf.fr/cryoEM-SOS

Courtesy of I. Kandiah

Users can submit proposals from **1st June 2023** (friendly users from July 2023)



<https://www.esrf.fr/CM01>

Courtesy of I. Kandiah

THANK YOU!

