https://cdn.rcsb.org/pdb101/molecular-machinery/

Synchrotron Radiation and Structural Biology

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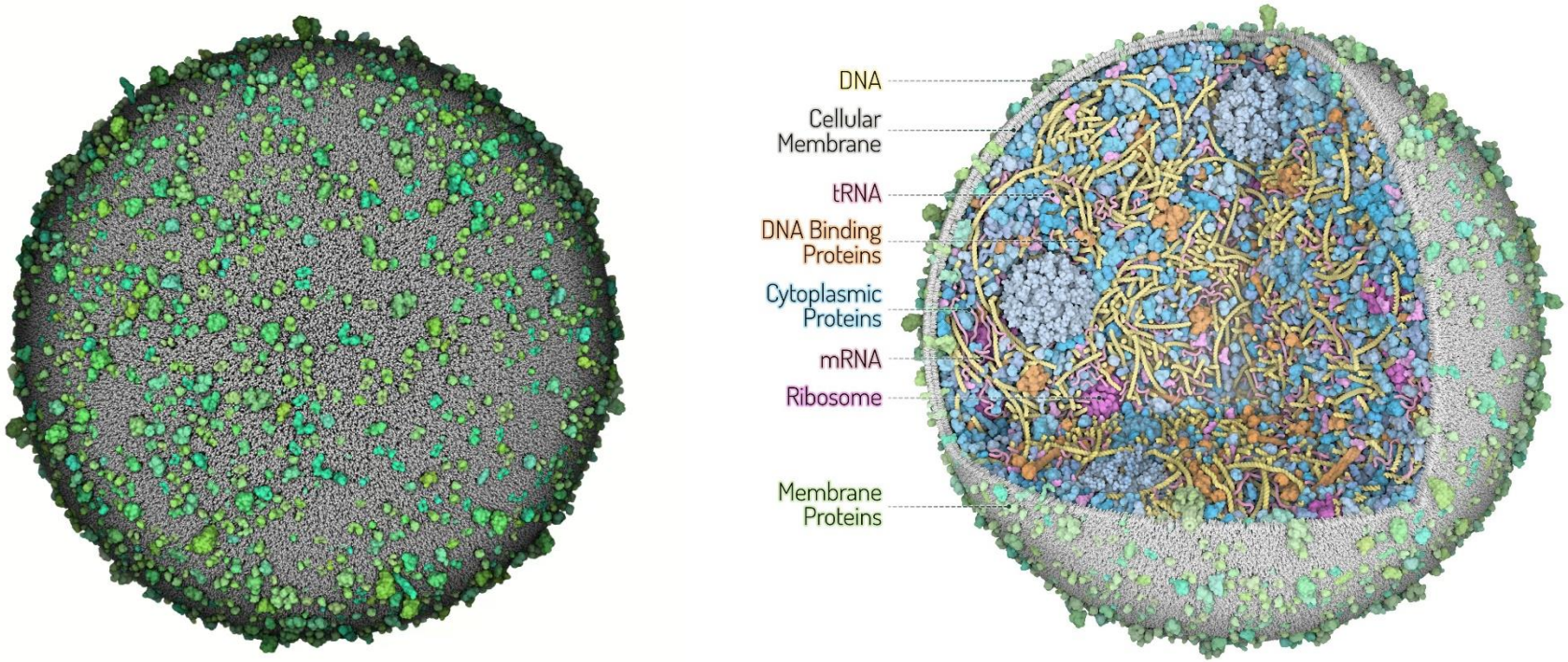
Structural Biology Group

Outline:

- What is Structural Biology?
- [Macromolecular] crystallography (MX)
- Why use Synchrotron Radiation for Structural Biology?
- Using Synchrotron Radiation for Structural Biology
- Landmarks in MX
- Radiation damage in MX @ 4th generation synchrotrons
- Structural Biology without crystals: SAXS, cryo-EM
- The future of MX @ SR: time-resolved/room-temperature serial crystallography
- The role of structural biology in the new biology
- In-house research

WHAT IS STRUCTURAL BIOLOGY

The human body is immensely complex, comprising trillions of cells, proteins and molecules that work in concert to keep all of our systems up and running. Each of these microscopic workers has a specific job; when these jobs aren't carried out correctly, diseases can result.

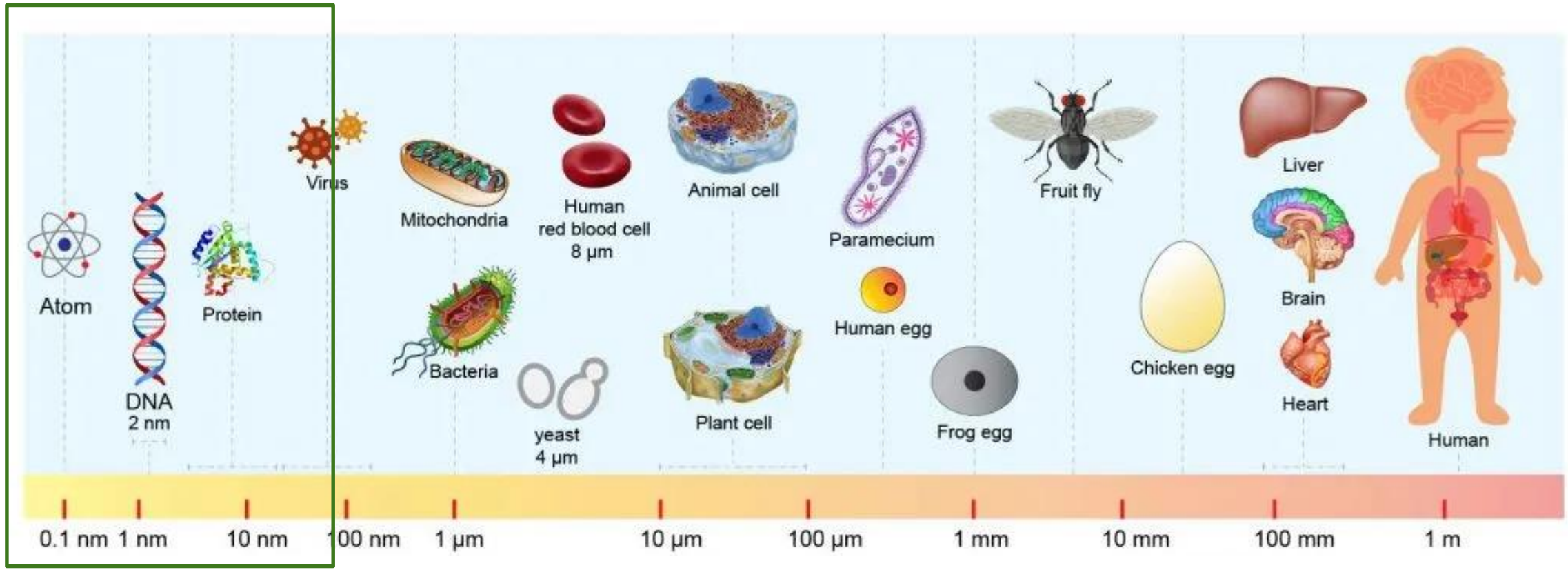


Mycoplasma cell at the beginning of its life cycle
M. Maritan, Scripps Research https://ccsb.scripps.edu/gallery/mycoplasma_model/

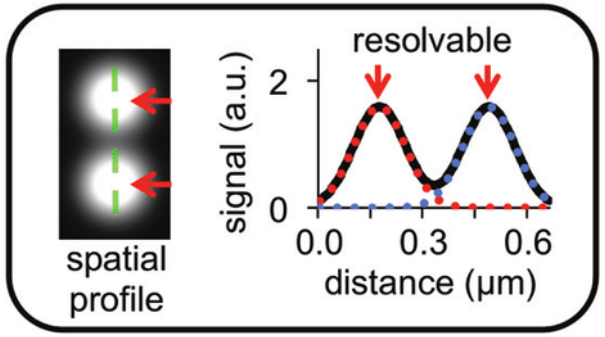
WHAT IS STRUCTURAL BIOLOGY

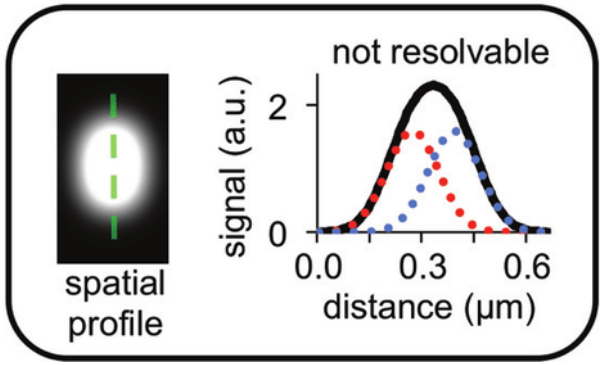
One approach scientists use to understand how these systems work is **structural biology**, which focuses on determining the architecture of biological molecules at atomic resolution

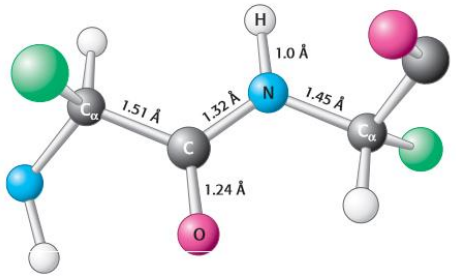
Central Dogma of Structural Biology: ‘Form = Function’

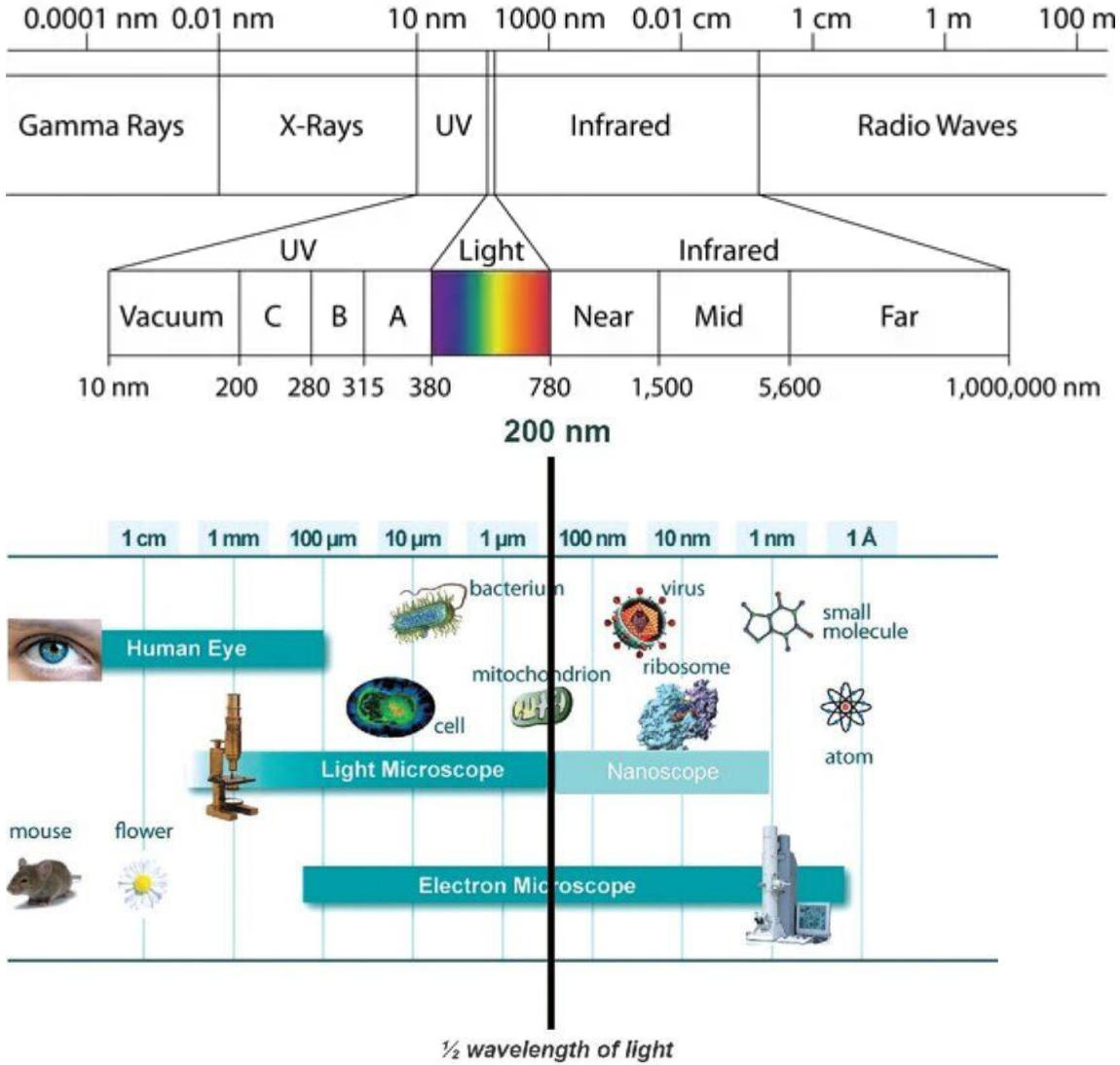


WHY WE NEED X-RAYS TO ACHIEVE ATOMIC RESOLUTION







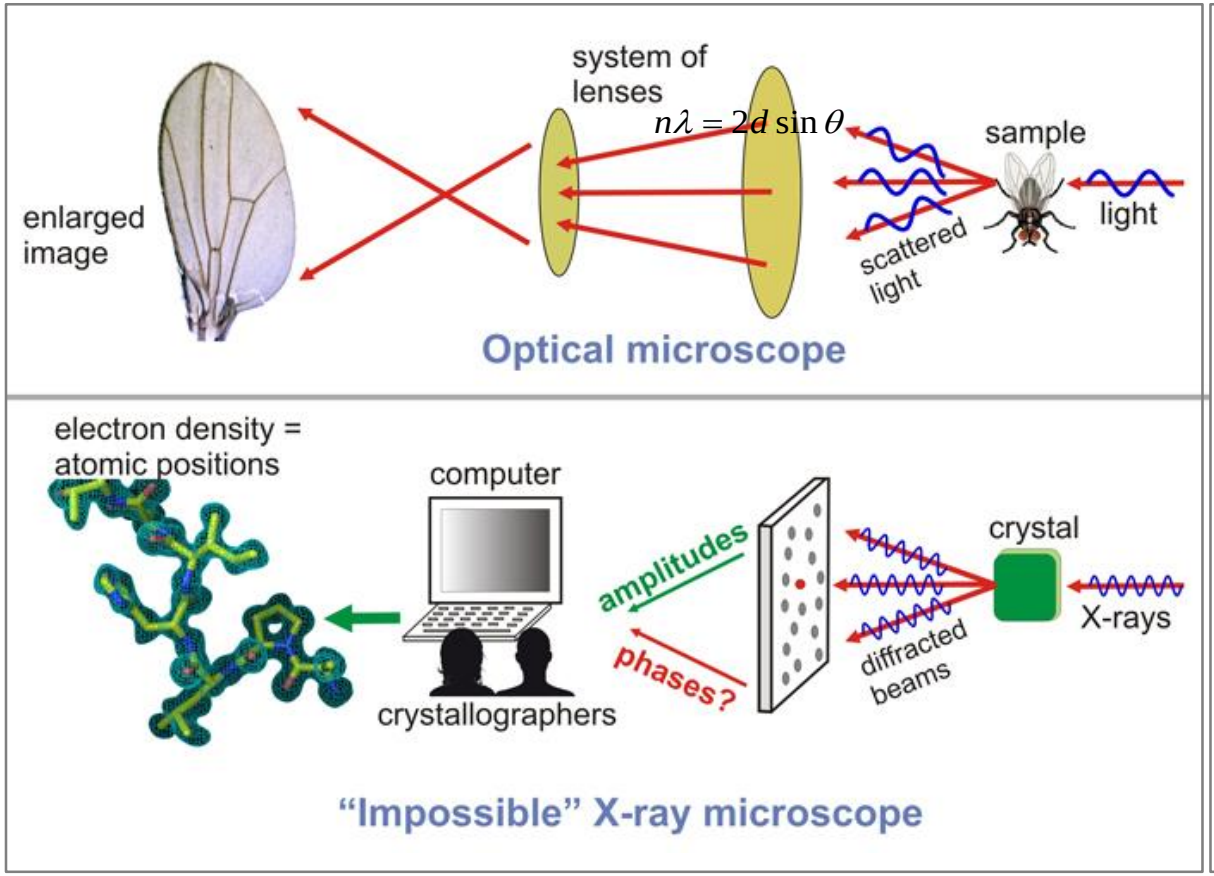


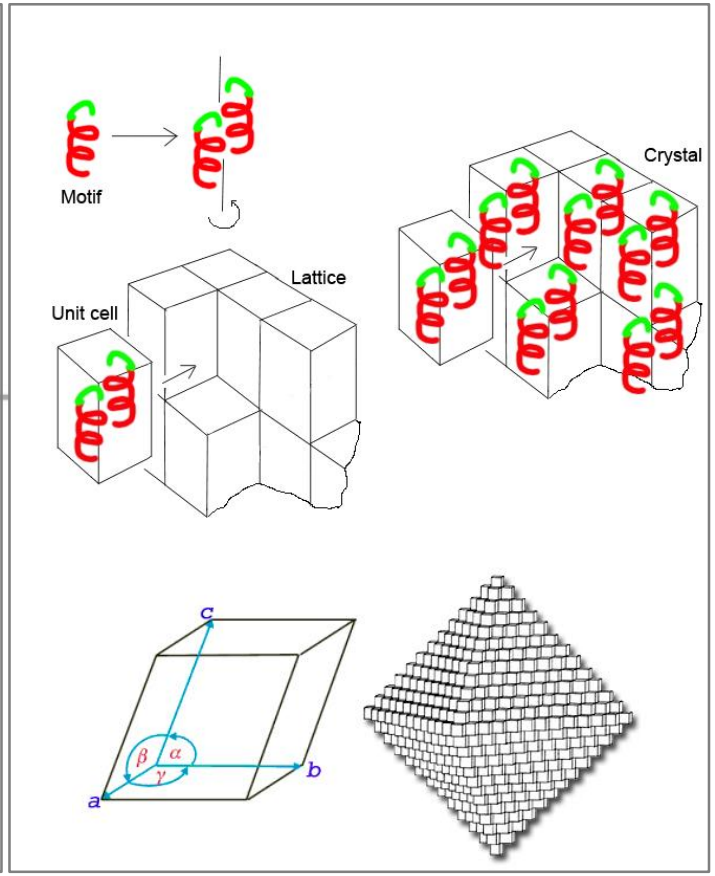
$$res_{max} = \lambda / 2$$

To see individual atoms in molecules need to use 0.5 Å > λ > 4.0 Å => X-rays

X-RAY CRYSTALLOGRAPHY

Visible light is refracted by lenses and can be focused.
X-rays are (mostly) not refracted/focused, but if we have a regular structure of the object (like a **crystal**) one can use **diffraction** (not a focused image)

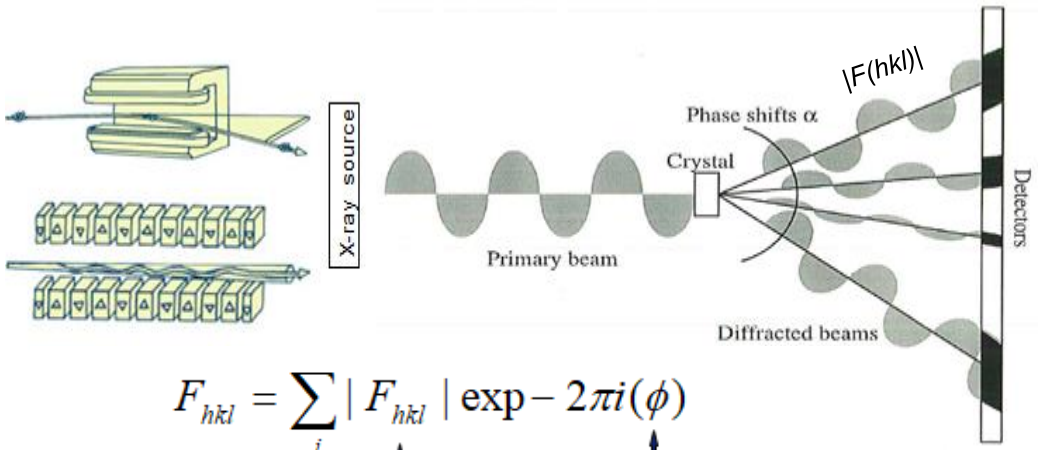




MACROMOLECULAR X-RAY CRYSTALLOGRAPHY (MX)

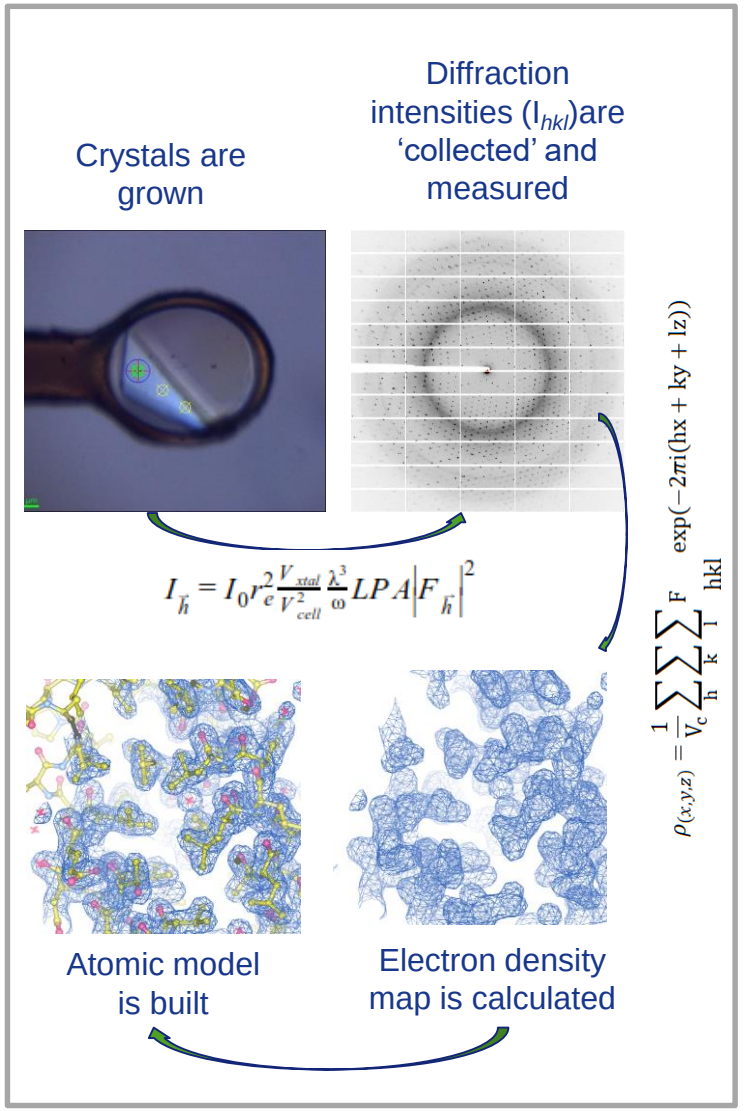
Diffraction data should suffice to define the atomic structure of a crystallized macromolecule

Nevertheless, it took decades before the pioneering efforts of **M. Perutz** and **J. Kendrew** provided the means to solve the problem of turning diffraction patterns into atomic coordinate sets. They received the 1962 Nobel Prize for their work on protein structure determination



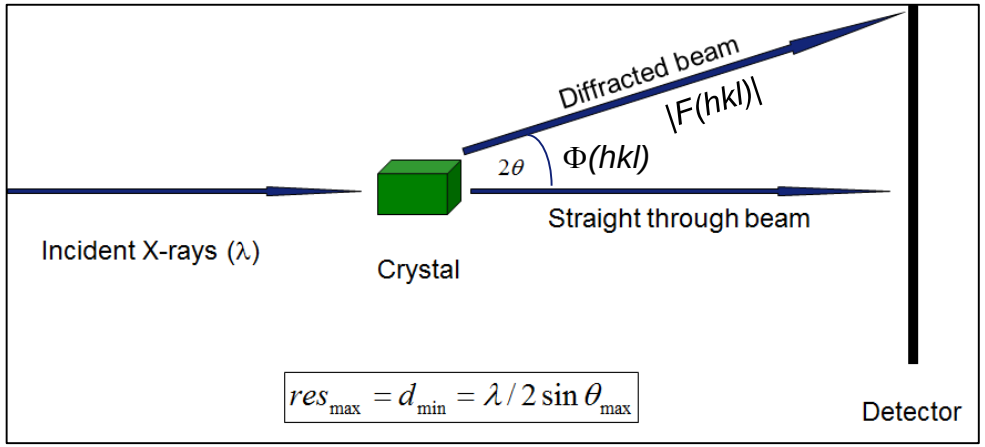
$$F_{hkl} = \sum_j |F_{hkl}| \exp -2\pi i(\phi)$$

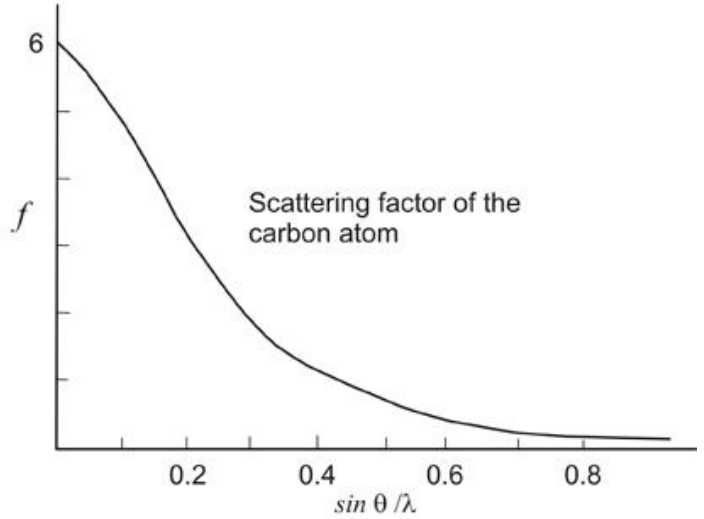
Amplitude — Phase



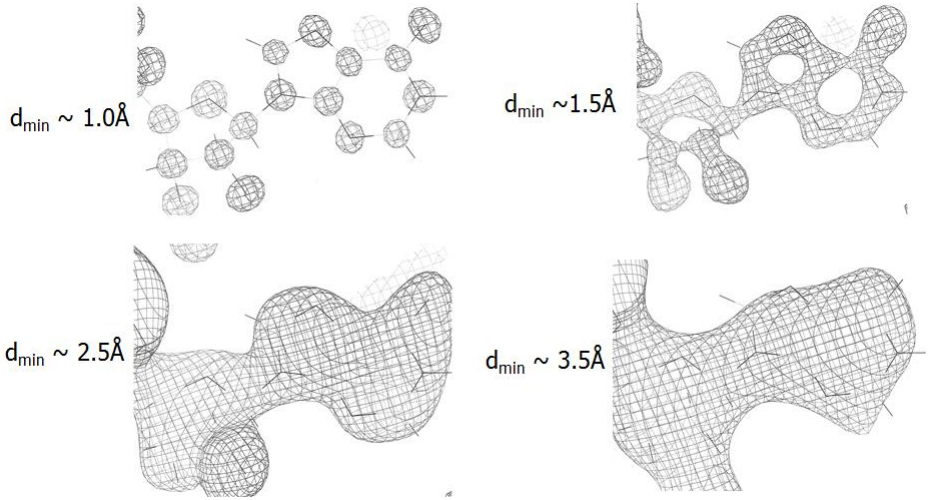
Result: atomic model of macromolecule of interest = atomic coordinates (x,y,z) and displacement factors (B, U_{ij})

ACHIEVING CLOSE TO ATOMIC RESOLUTION FROM CRYSTALS OF BIOLOGICAL MACROMOLECULES IS NOT EASY





$$I_{hkl} = I_0 r_e^2 \frac{V_{xtal}}{V_{cell}^2} \frac{\lambda^3}{\omega} LPA|F_{hkl}|^2$$

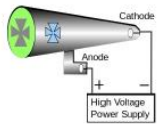


- For a crystal of a given size, energy of a diffracted beam is:
  - proportional to incident X-ray beam
  - proportional to volume of crystal
  - inversely proportional to square of the unit cell volume

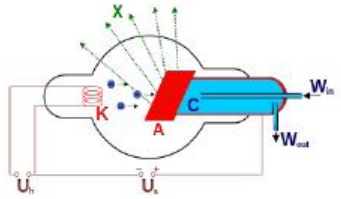
  → for crystals of macromolecules (large unit cells, small volumes) we **need intense sources of X-rays** to get maximum information (i.e. data resolution).

X-RAYS SOURCES

Crookes (1869)
(cold cathode)
Ionization → e- from cathode to anode

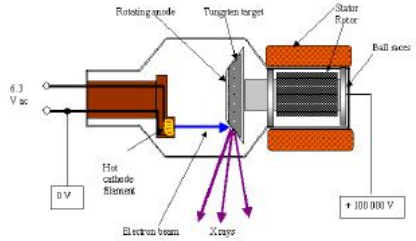


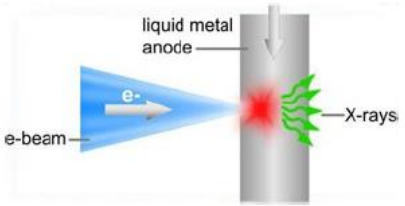
Coolidge (1917)
(hot cathode)
Cathode: heated filament



Moving target
Cathode: heated filamen
Anode: rotating/liquid

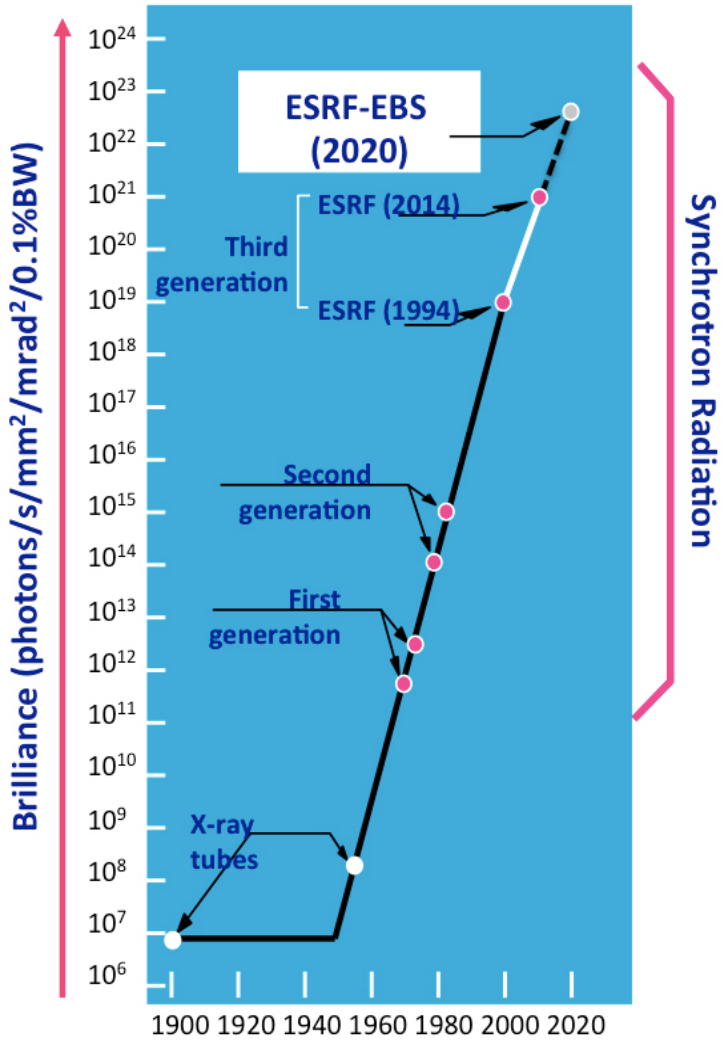
Siemens, 1933





Increasing X-ray Power

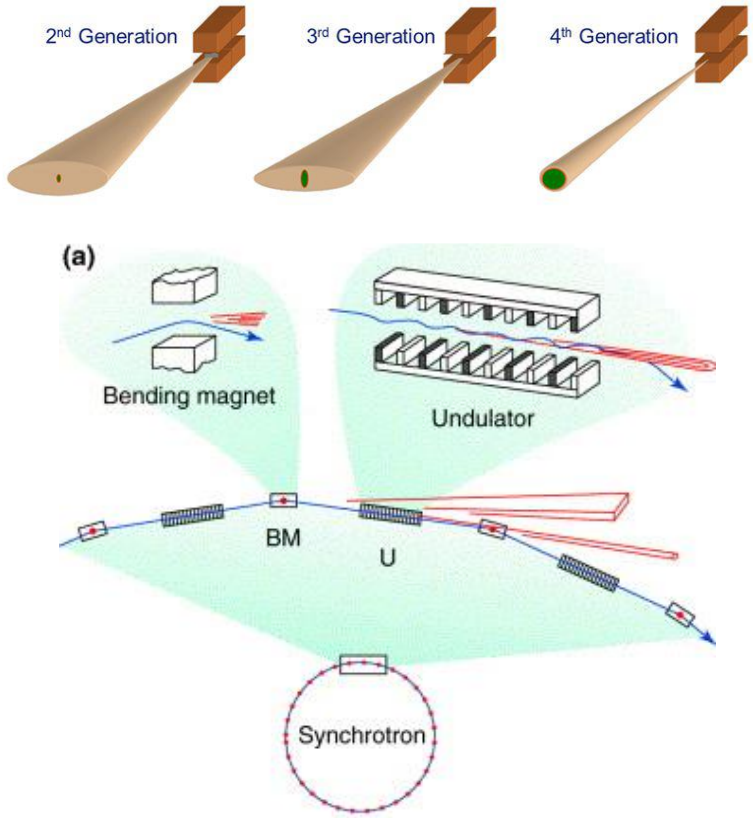
Intensity + collimation =
'brilliance'



SYNCHROTRON RADIATION (SR)

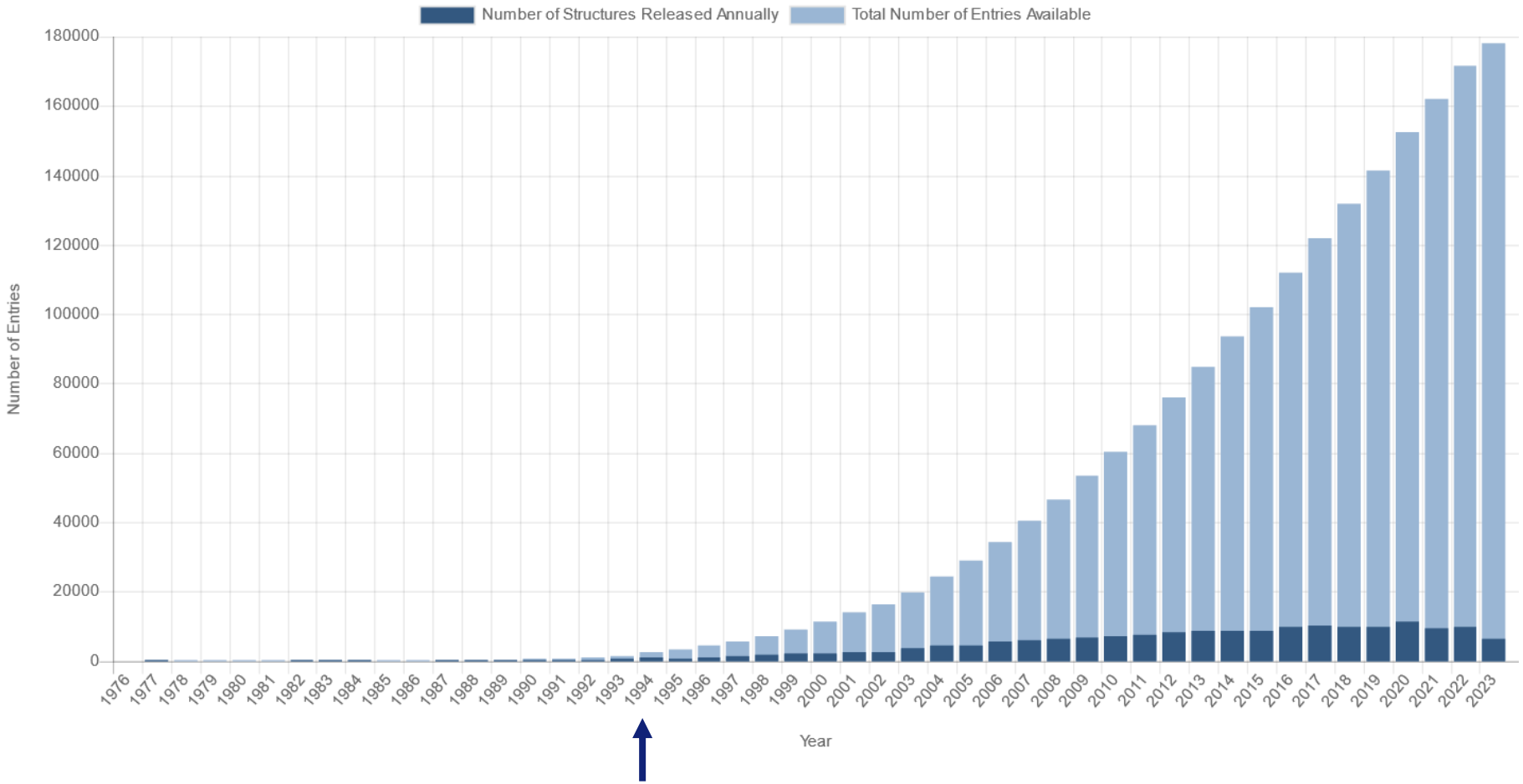
Synchrotron radiation was seen initially as a costly waste product in the design of accelerators for high-energy physics experiments, but it was realised later that synchrotron radiation could, in principle, compete favorably with conventional X-ray tubes for X-ray studies.

This opportunity came to the attention of biological crystallographers with the report, published in 1971, of diffraction experiments conducted on muscle at the DESY synchrotron in Hamburg. The interest then focused on the prospect of greater X-ray fluxes; later on, other properties of synchrotron radiation have been exploited as well, including its continuous spectrum and definite time structure.



- Brilliance at 4th generation sources
  - small, high flux X-ray beams (better S/N from smaller samples)
- Wavelengths from IR to hard X-rays
  - Anomalous dispersion, fluorescence techniques
- Time structure (ps)
  - Time resolved studies
- Multiscale (mm → μm → nm)

THE PROTEIN DATABANK (PDB: HTTPS://WWW.RCSB.ORG/)



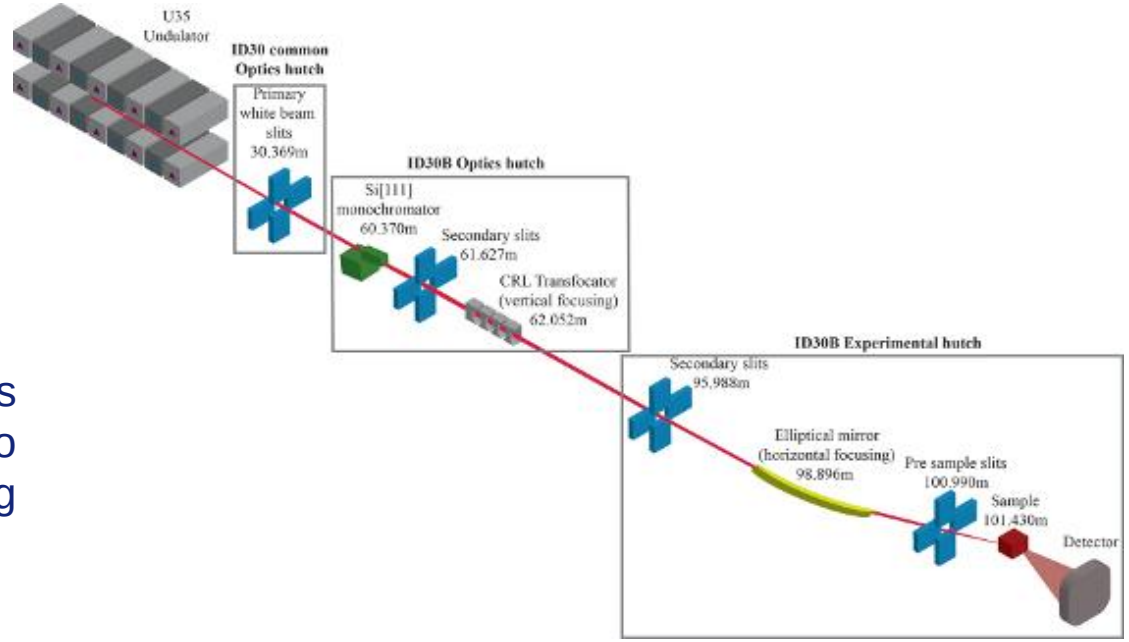
First MX experiment at the ESRF

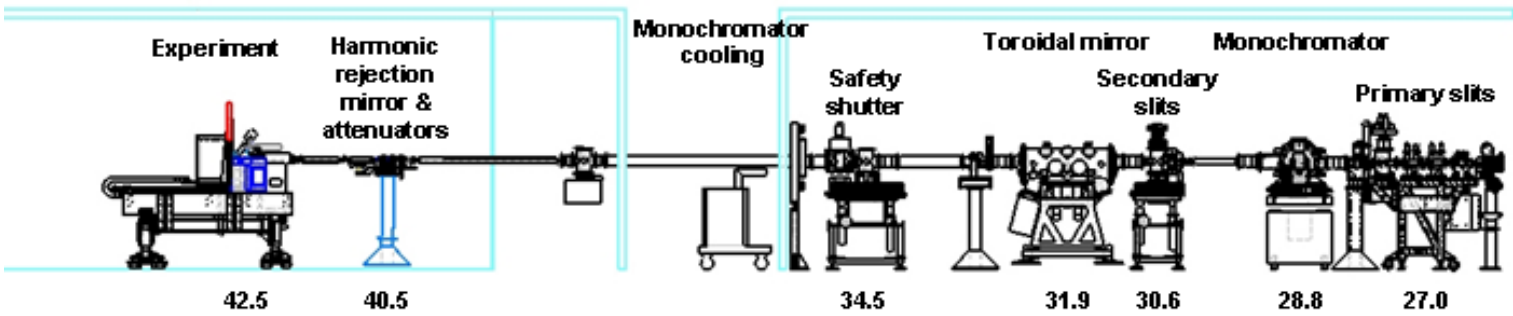
MX AT SYNCHROTRON SOURCES

Optimised SR gives a bright, highly collimated source of X-rays that allow the study of weakly diffracting macromolecule crystals, enabling smaller crystals and/or crystals with large unit cells

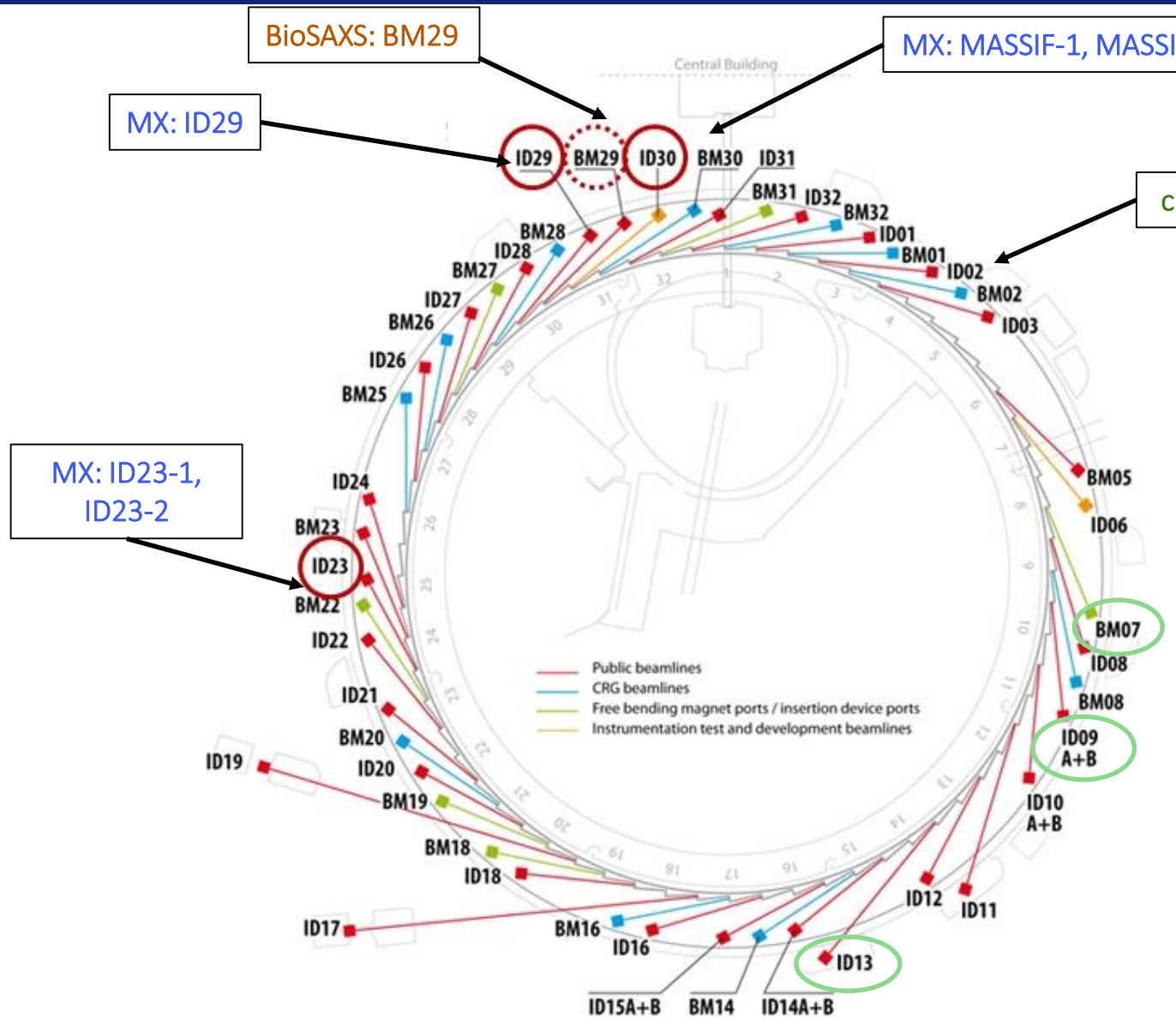
A wavelength of 0.98 Å is most commonly used with those between 0.6 - 2.0 Å (20 - 6 keV) usually accessible

The tunability of the radiation allows wavelengths to be precisely selected to exploit the anomalous scattering for phasing in structure determination





THE STRUCTURAL BIOLOGY BEAMLINES AT THE ESRF



Also:

- ID13
- ID09
- FiP (BM30A → BM07) (IBS)

Operated by ESRF-EMBL Joint Structural Biology Group

THE STRUCTURAL BIOLOGY BEAMLINES AT THE ESRF

X-ray Macromolecular crystallography

With EMBL European Molecular Biology Laboratory

- **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 Institut de Biologie Structurale

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

Small angle X-ray scattering

- **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, NEUTRONS FOR SOCIETY

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

Complementary methods

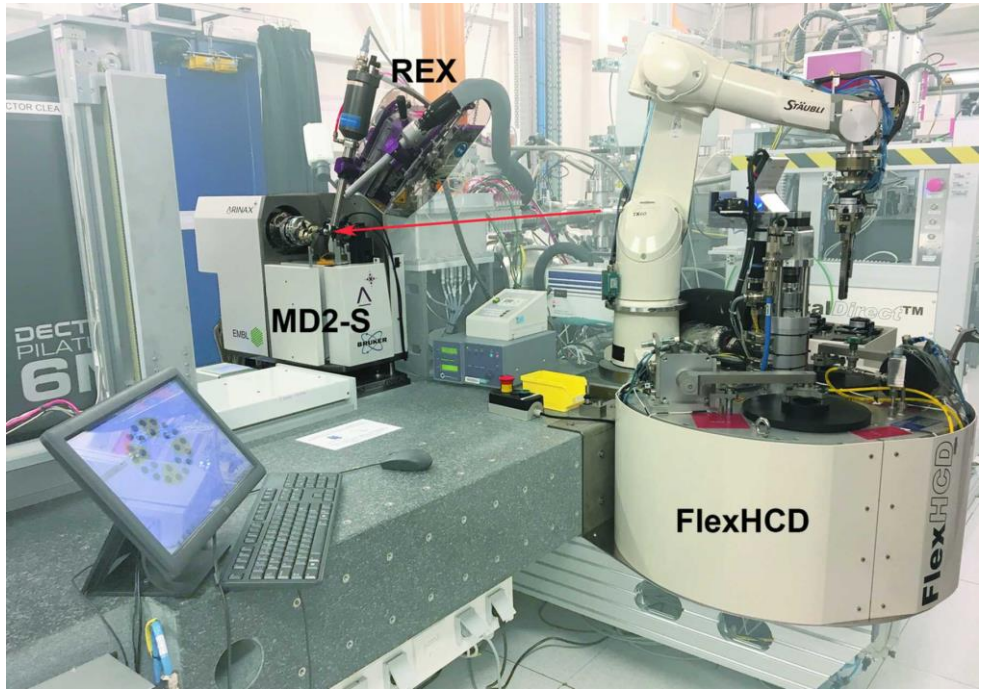
With ibs Institut de Biologie Structurale

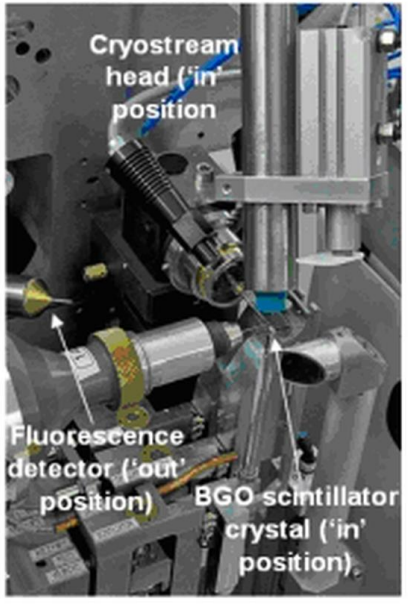
- **icOS**: *In crystallo* optical spectroscopy: UV/Vis absorption, fluorescence, Raman

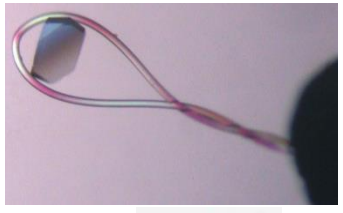
With cea GRENOBLE

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

THE EXPERIMENTAL SETUP OF AN MX BEAMLINE



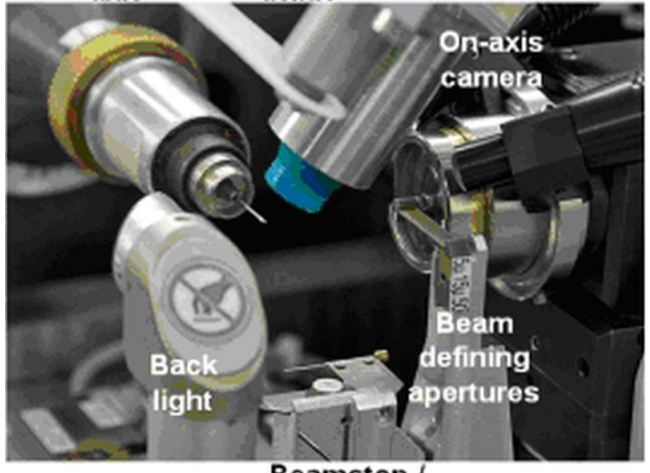




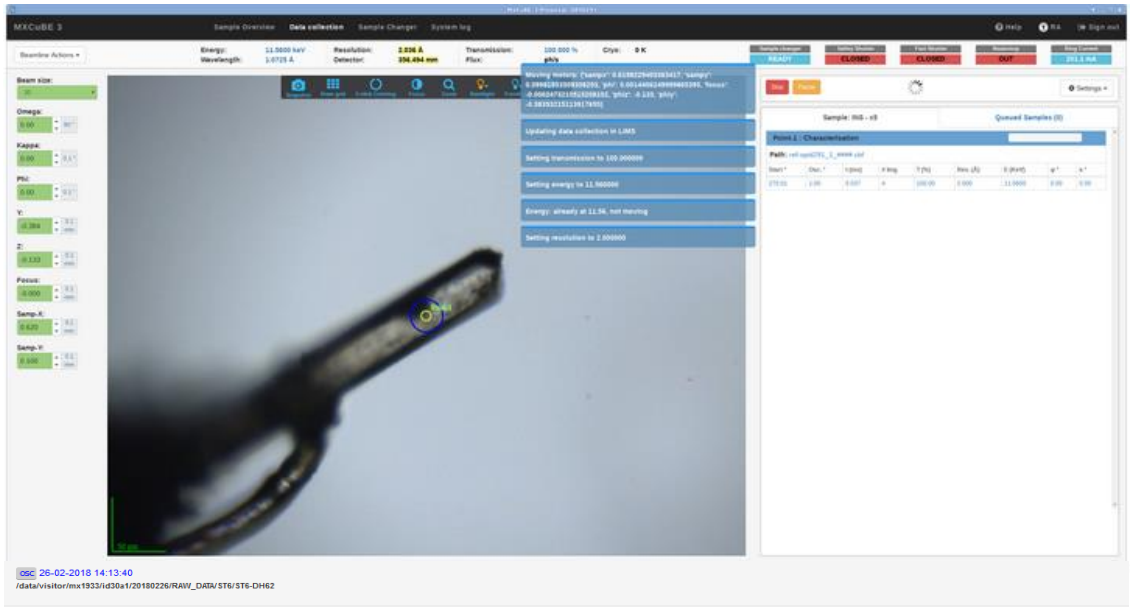
FlexHCD sample changers: 24 slots

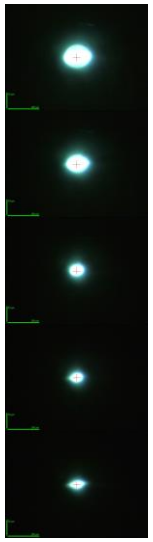
Unipuck:192 samples

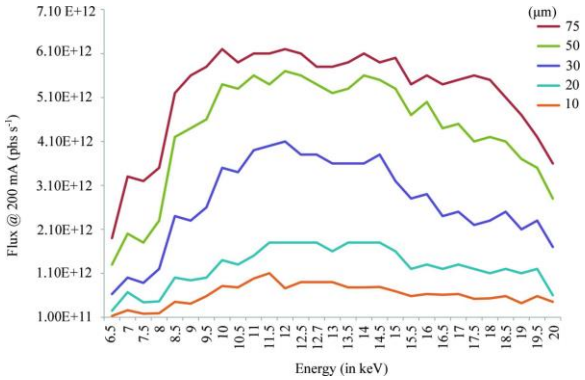
SC3:120 samples

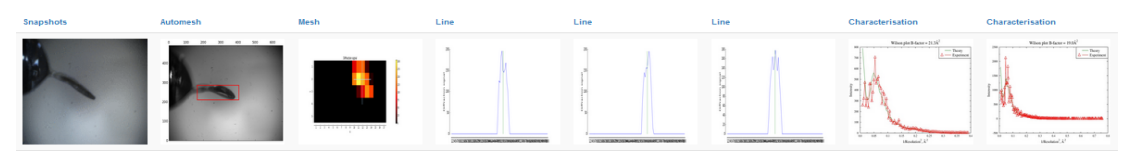


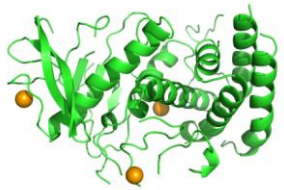
MX AUTOMATION: USER FRIENDLY, ON-LINE DATA ANALYSIS

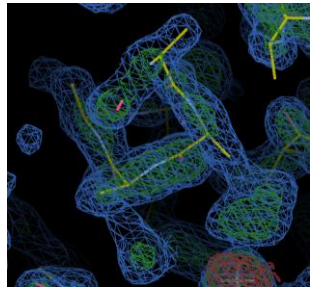


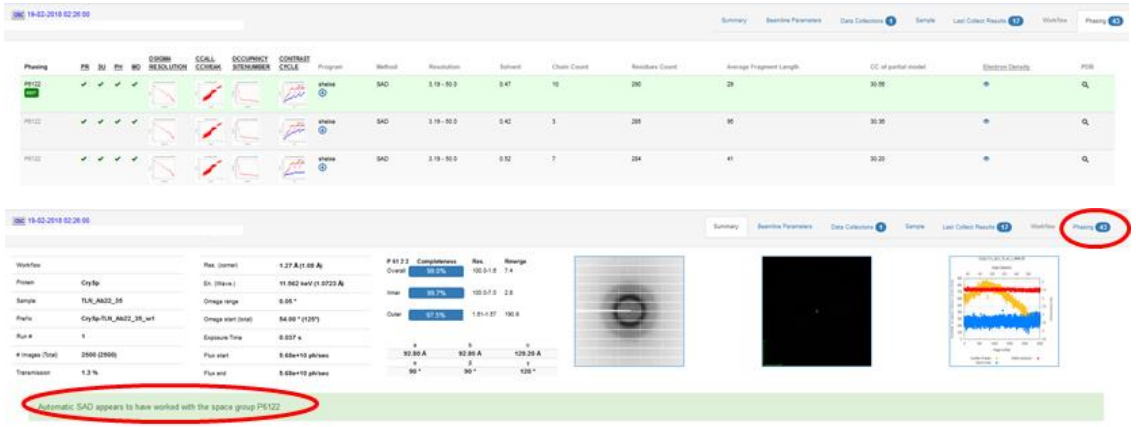


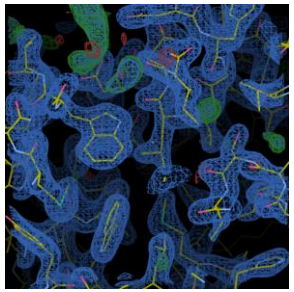


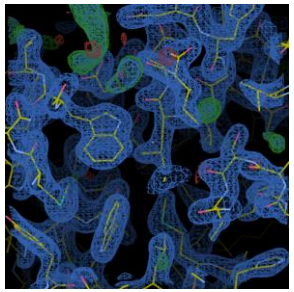








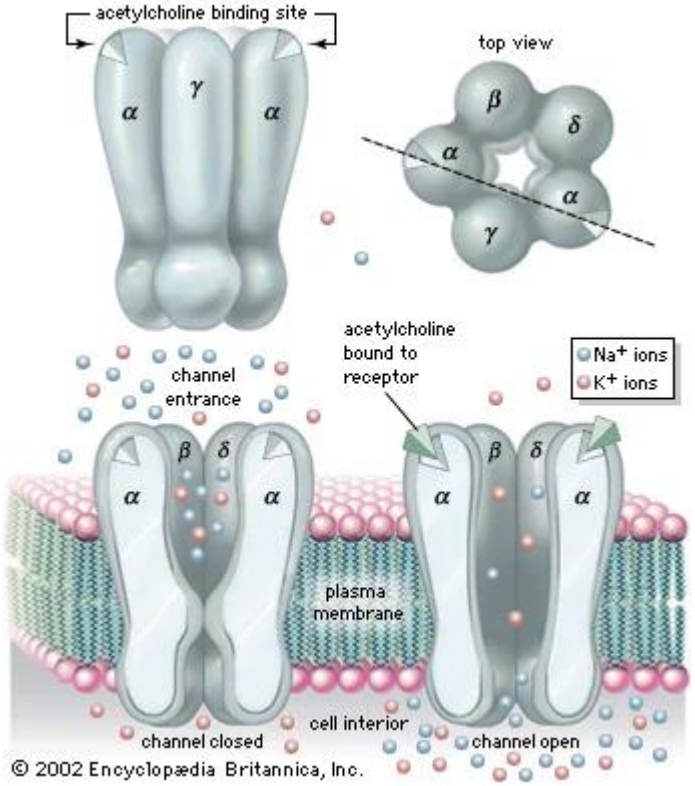


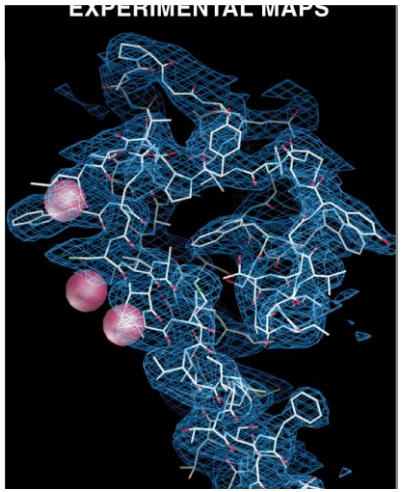


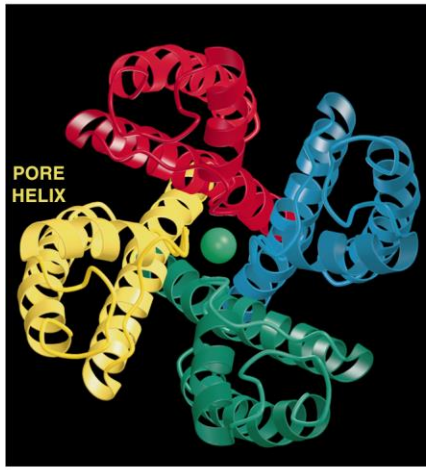
LANDMARKS IN MX – THE CRYSTAL STRUCTURES OF CELL ION CHANNELS

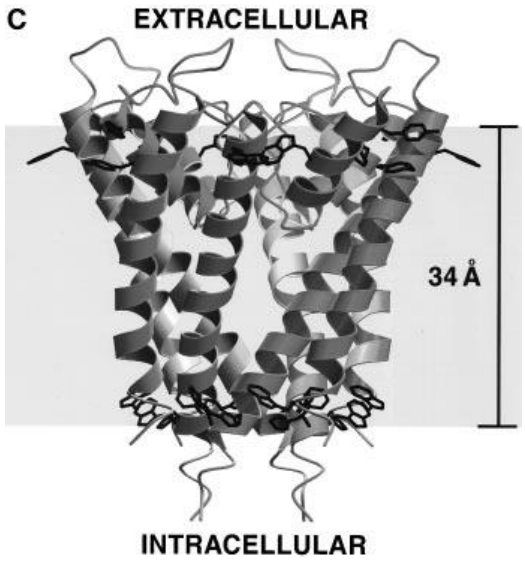
R. MacKinnon was awarded with the Nobel Prize for Chemistry in 2003 for his pioneering research on ion channels in cell membranes

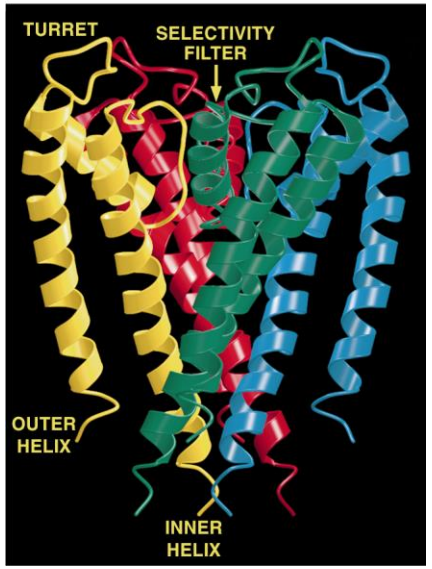
Ion channels control the pace of the heart, regulate the secretion of hormones and generate electrical impulses in the nervous system







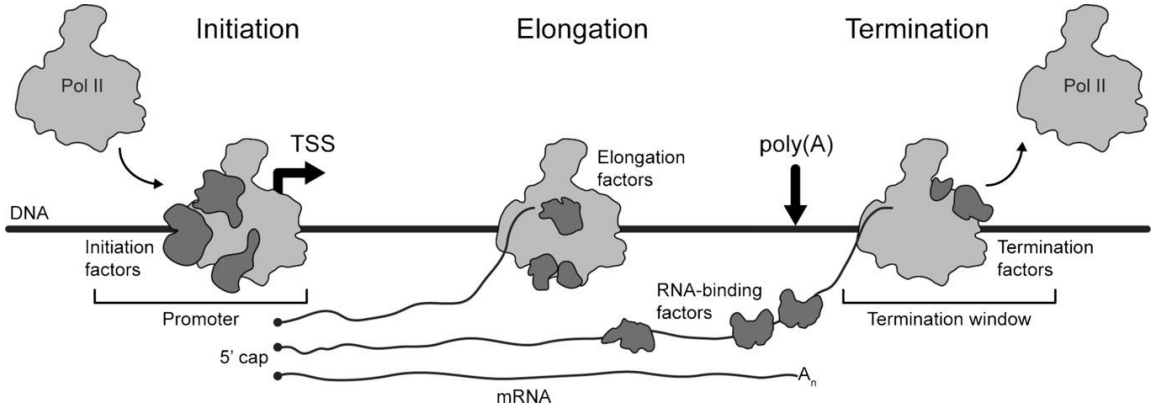




LANDMARKS IN MX – THE CRYSTAL STRUCTURES OF TRANSCRIPTION ENZYMES

R. Kornberg was awarded with the Nobel Prize for Chemistry in 2006 for his studies of the molecular basis of eukaryotic transcription

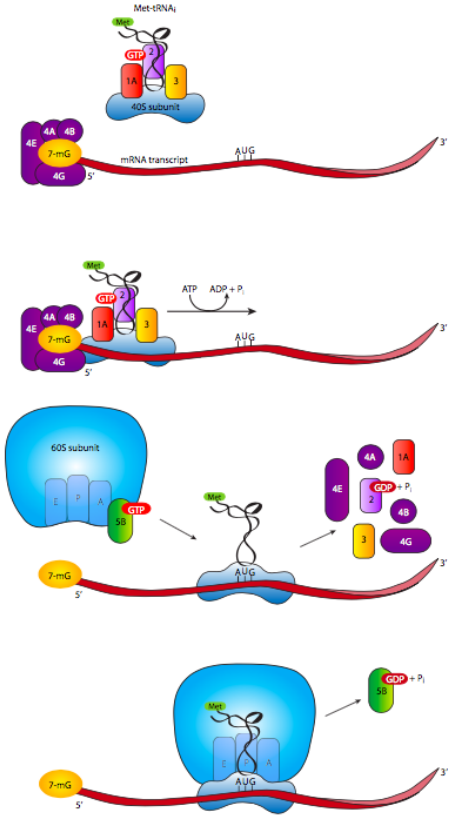
RNA polymerase II is a multiprotein complex that transcribes DNA into RNA. It is one of the three RNAP enzymes found in the nucleus of eukaryotic cells. A 550 kDa complex of 12 subunits. A wide range of transcription factors are required for it to bind to upstream gene promoters and begin transcription

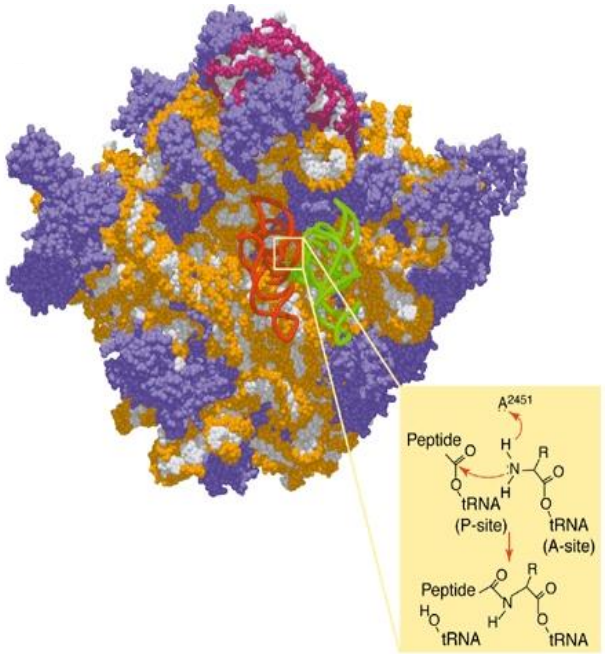


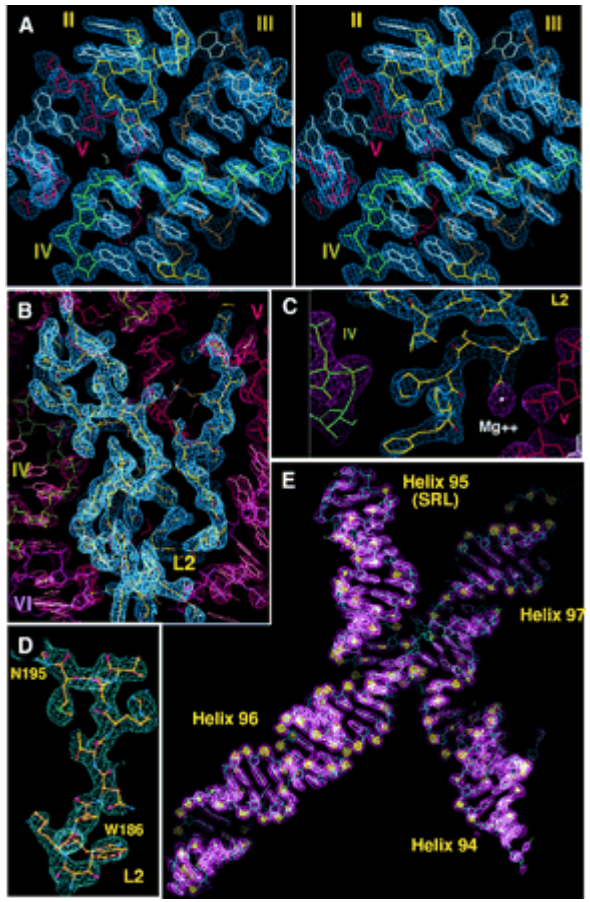
LANDMARKS IN MX - THE CRYSTAL STRUCTURE OF THE RIBOSOME

V. Ramakrishnan, **T. A. Steitz** and **A. E. Yonath** were awarded with the Nobel Prize for Chemistry in 2009 for studies of the structure and function of the ribosome

Ribosomes are macromolecular machines that carry out protein synthesis (mRNA translation). Ribosomes consist of a small and large ribosomal subunits. Each subunit consists of RNA molecules and many ribosomal proteins. The ribosomes and associated molecules are also known as the translational apparatus



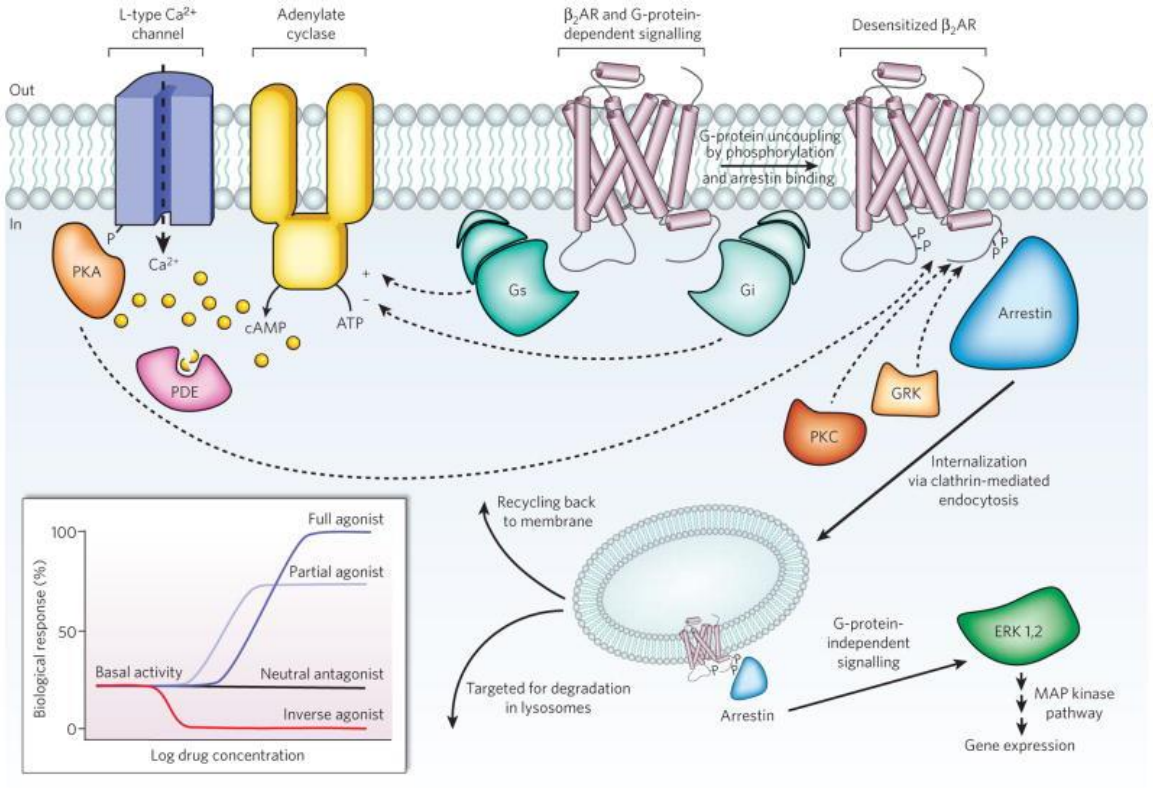


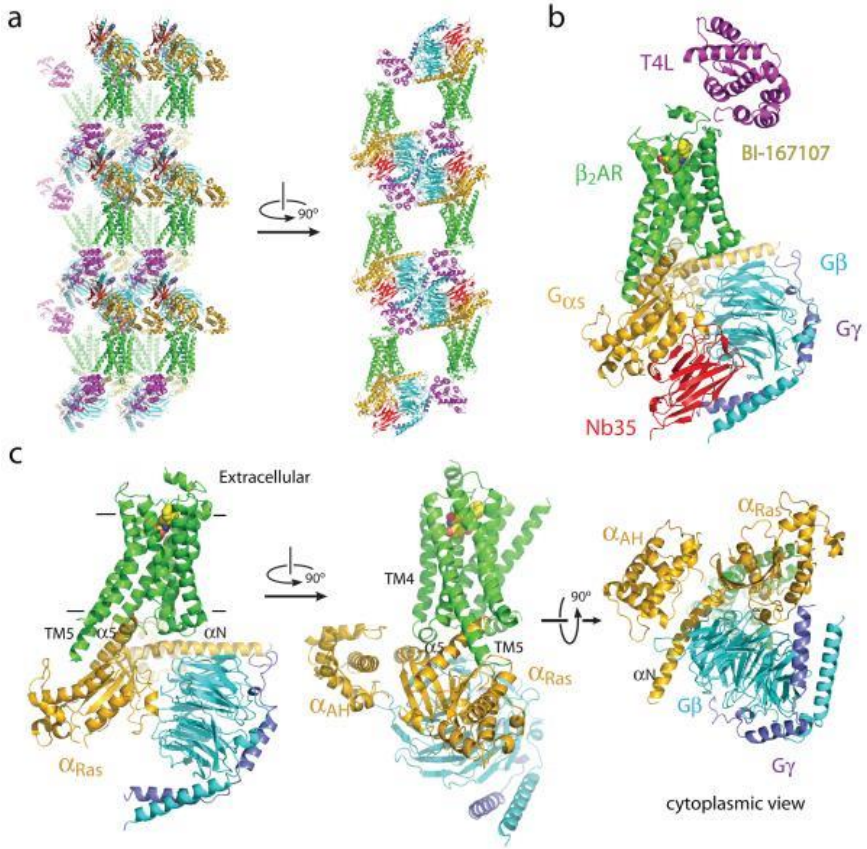


LANDMARKS IN MX – THE CRYSTAL STRUCTURE OF A MEMBRANE RECEPTOR

R. Lefkowitz and **B. Kobilka** were awarded with the Nobel Prize in 2012 for studies of G-protein-coupled receptor signalling G-protein-coupled receptors (GPCRs)

GPCRs are cell surface receptors that mediate most of our physiological responses to hormones, neurotransmitters and environmental stimulants





RADIATION DAMAGE IN MX

Global radiation damage

- Degradation of diffraction properties of a crystal as a function of absorbed dose.

  ↘ Resolution (Å)

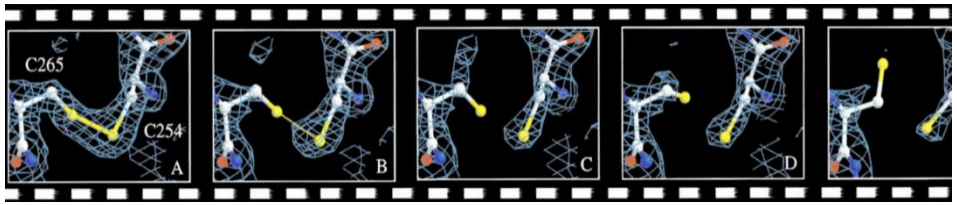
- Before 1990: **room temperature** crystallography requires data from several crystals to obtain a complete dataset

- 1990s: **cryo-crystallography** extends the lifetime of crystals to be able to record a full dataset (or more) on one single crystal (Garman & Schneider, *J. Appl. Cryst.* (1997))

- **Even at cryo-temperatures maximum absorbed dose/crystal ~20 - 30MGy**

Specific radiation damage

- 2000s: 3rd generation synchrotron sources revealed damage in the **real space**, on specific chemical groups

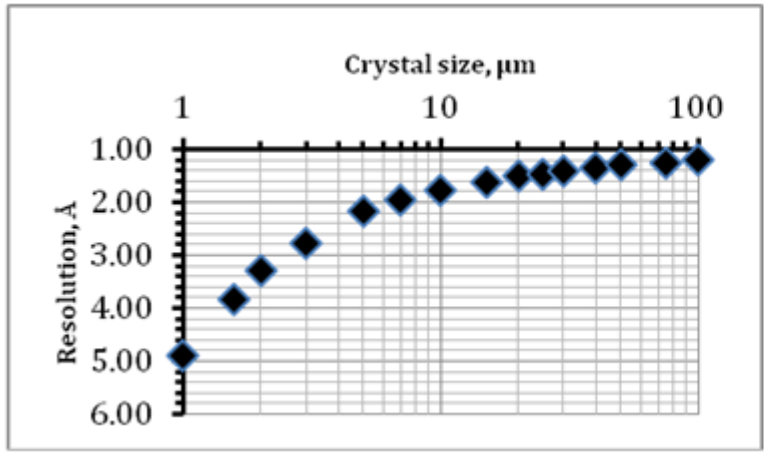


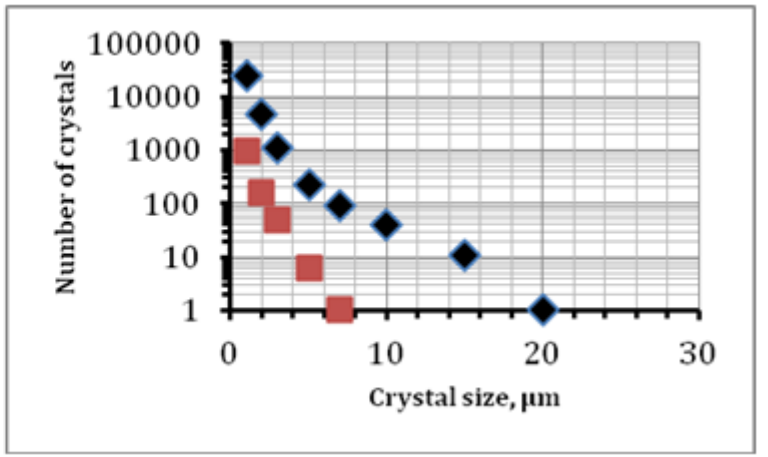
Adapted from Weik *et al.*, *PNAS* (2000)

- **Specific** radiation damage is a concern in crystallography at **cryogenic temperature**

- The onset of **specific** damage is faster than **global** damage at **cryogenic temperature**

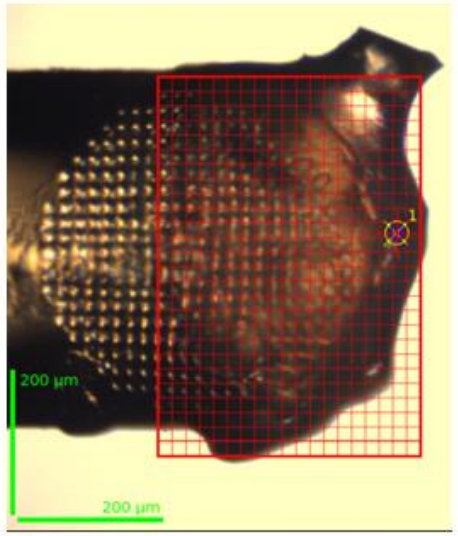
GLOBAL RADIATION DAMAGE, CRYSTAL SIZE AND DATA SET RESOLUTION

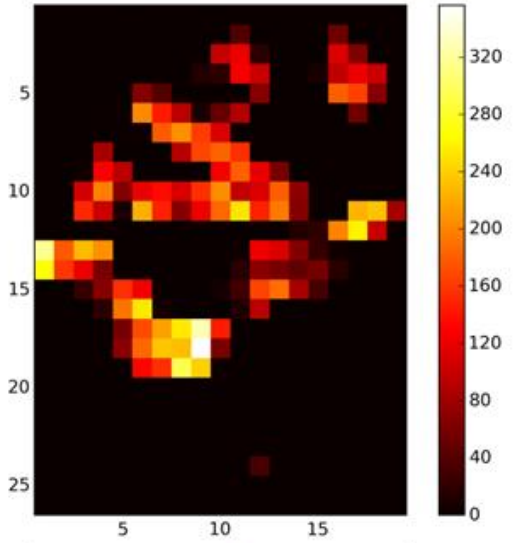


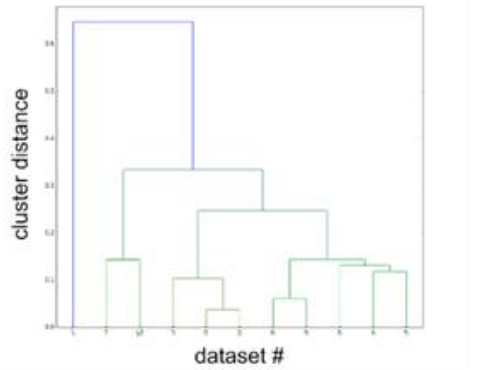


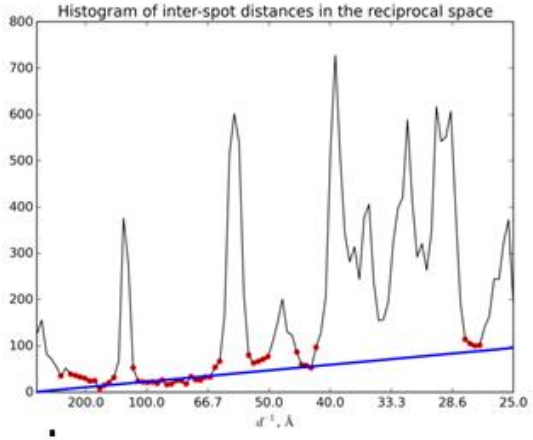
- The resolution of a *complete* diffraction dataset (i.e. symmetry unique reciprocal lattice points) that can be collected from a crystal of a biological macromolecule is limited by radiation damage.
- For small crystals, the merging of partial data sets from many crystals will be required in order to collect a complete data set at moderate resolution.
- New (old) paradigm for MX: **Multi-crystal data collection**

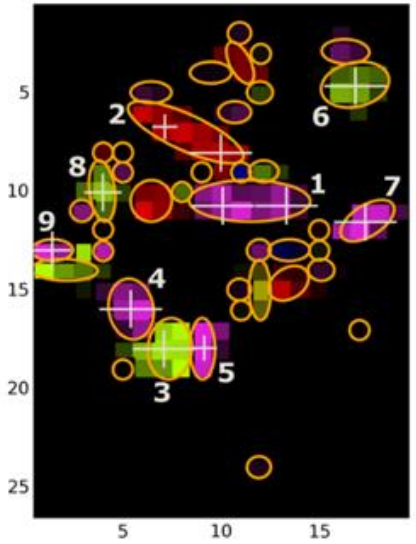
MULTI-CRYSTAL DATA COLLECTION WORKFLOW: MESH AND COLLECT

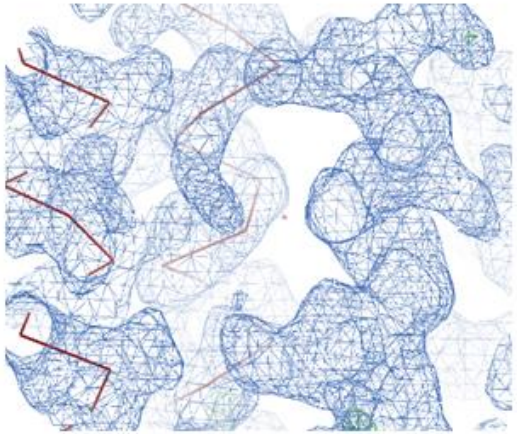








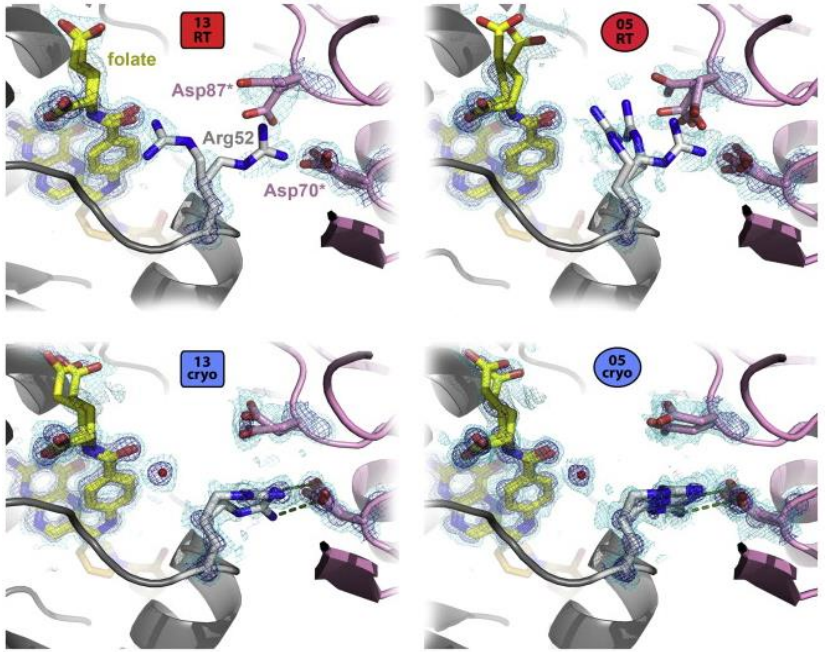




Melnikov et al. (2018). Acta Cryst. D74 (Pt 4):355-365

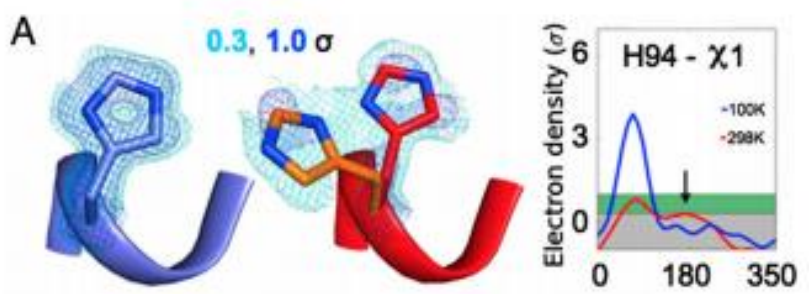
ROOM TEMPERATURE DATA COLLECTION

- Cryo-MX was one of the keys for success of structural biology, but
  - Cryo-structures do not display the same range of conformations as the RT structures.
  - They might hide functional conformations and prevent binding of substrates or inhibitors
- RT temperature crystal structures reveal physiologically relevant conformations “hidden” at 100 K
  - Present thermal motion closer to “native” conditions
  - Better interpretation of crystal structures, including for the design of new therapeutic agents



- **Hydrated microcrystals at room temperature can:**
  - **be activated**
  - **be soaked**
  - **We can carry out (and follow) reactions in them**

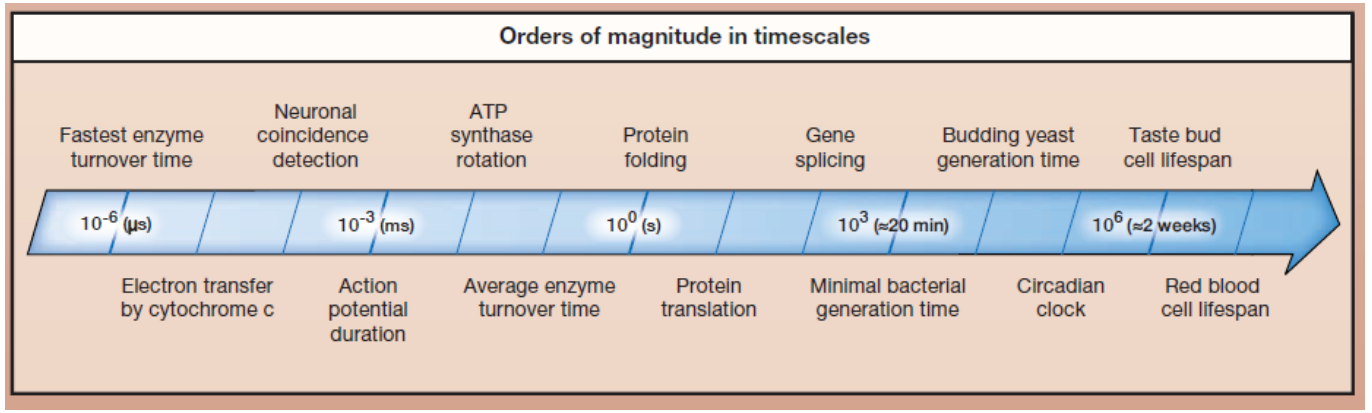
- Because of radiation damage, **serial crystallography** is the most valuable route to obtaining RT structures.



Fraser et al., PNAS 2011

MOVING TOWARDS AN UNDERSTANDING OF CONFORMATIONAL VARIABILITY/DYNAMICS

Single structure/data point doesn't give the full picture. Move towards ensembles of structures and molecular movies of molecules in action: **time-resolved serial crystallography**

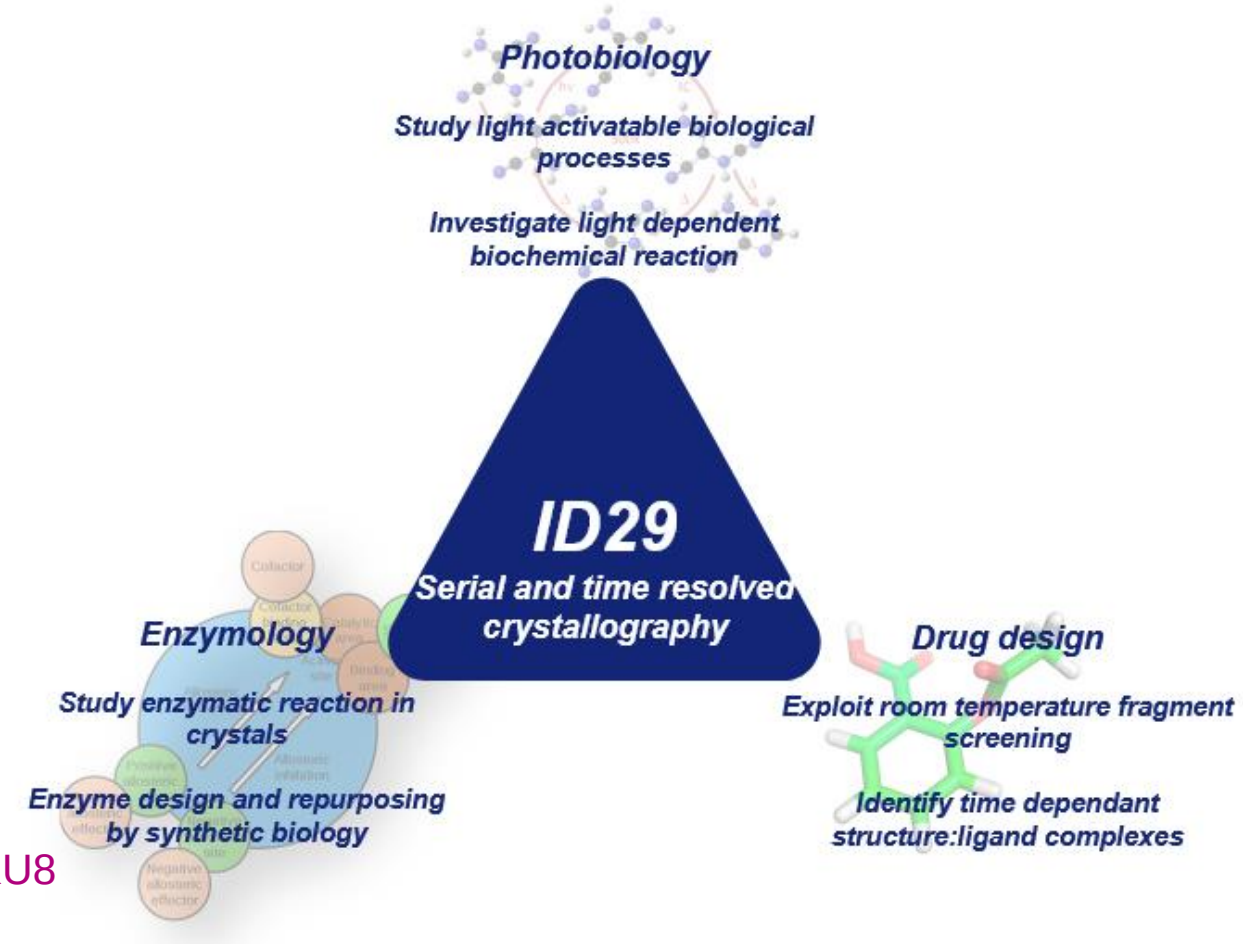


- Optimising the acquisition time in the micro-to-millisecond time range allows to study a vast majority of enzymatic processes
- 3rd generation synchrotrons are mostly limited to milliseconds due to detector and available flux at the sample position
- 4th generation allows for a x10 flux and $x10^5$-10^6 flux density

Time-resolved serial crystallography (TR/RT SSX)

- ✓ Room temperature serial crystallography
- ✓ Extremely high flux with exposure time in µs range and high repetition rate
- ✓ Tunable over a large energy range
- ✓ Accurate control timing system to trigger events
- ✓ Optimized sample consumption
- ✓ Adapted to different sample environments and crystal delivery systems
- ✓ Equipped with sample preparation lab and data analysis area

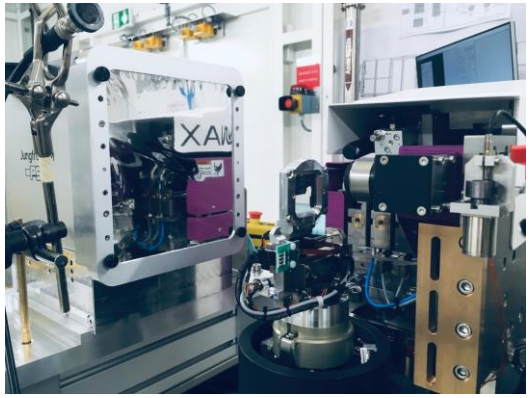
ID29 webinar: https://www.youtube.com/watch?v=C27tHuV9KU8



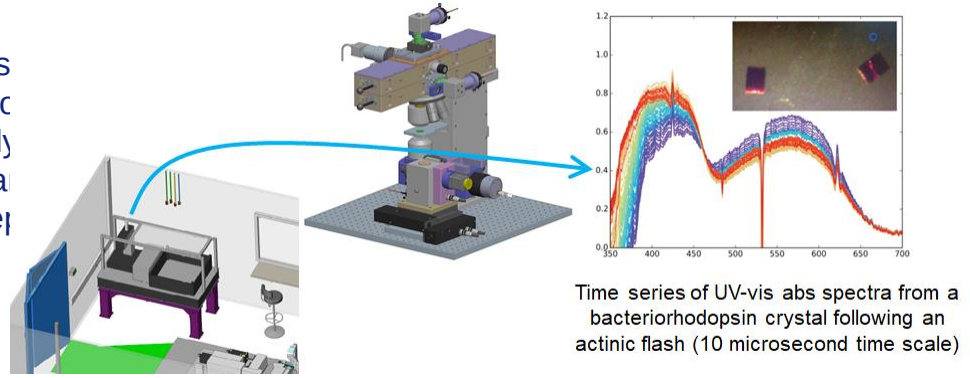
ID29 BEAMLINE- EXPERIMENTAL SETUP

A new ID29 optical layout was designed to tackle this time resolution, with support laboratory facilities and in synergy with *in crystallo* spectroscopy

Energy	10-20 keV
Bandwidth	0.4% and 1% (ΔE/E)
Flux	10^{15} - 10^{16} ph/s
Beamsize	4 x 2 μm²



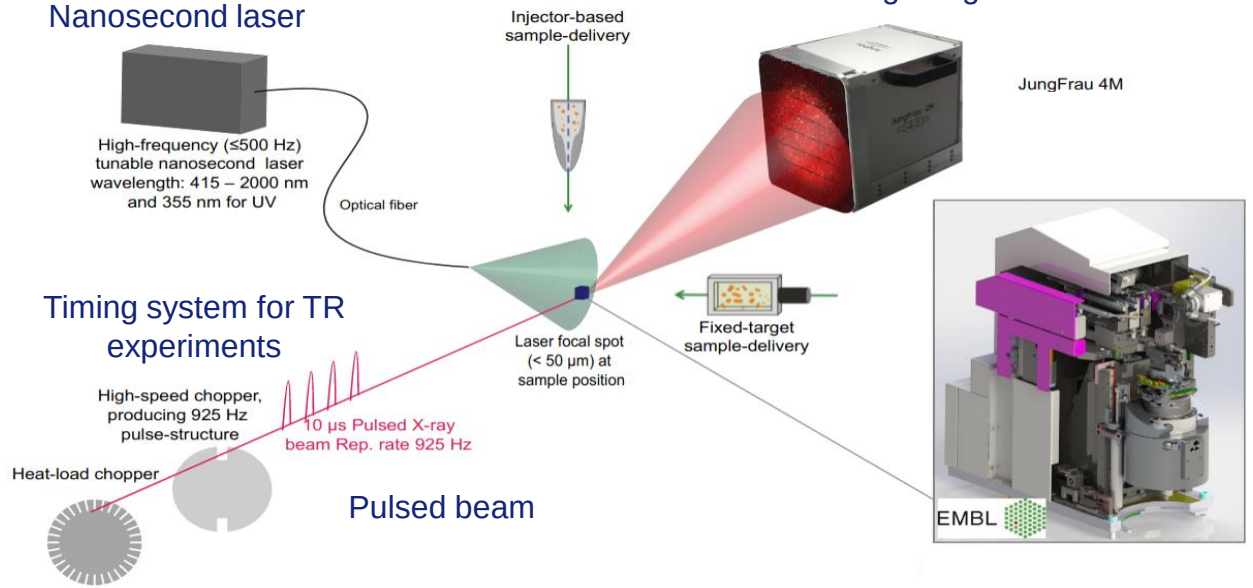
- Multiple s
- Time resc
- Extremely
- Microbea
- Since Sep

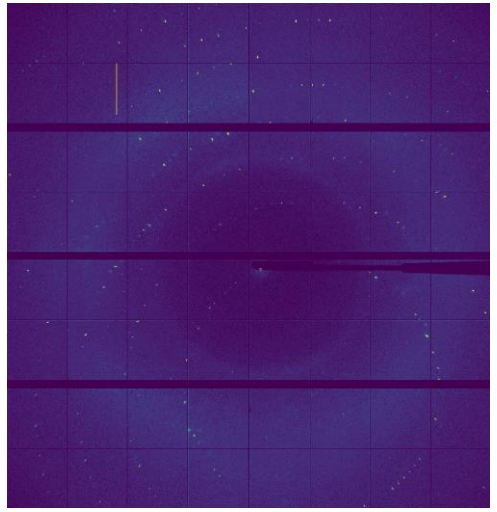


Time series of UV-vis abs spectra from a bacteriorhodopsin crystal following an actinic flash (10 microsecond time scale)

New integrating detector

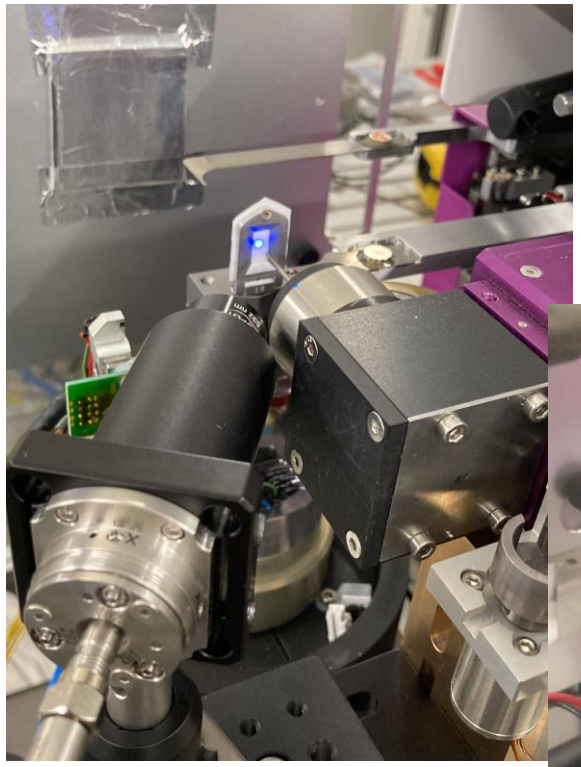
Nanosecond laser



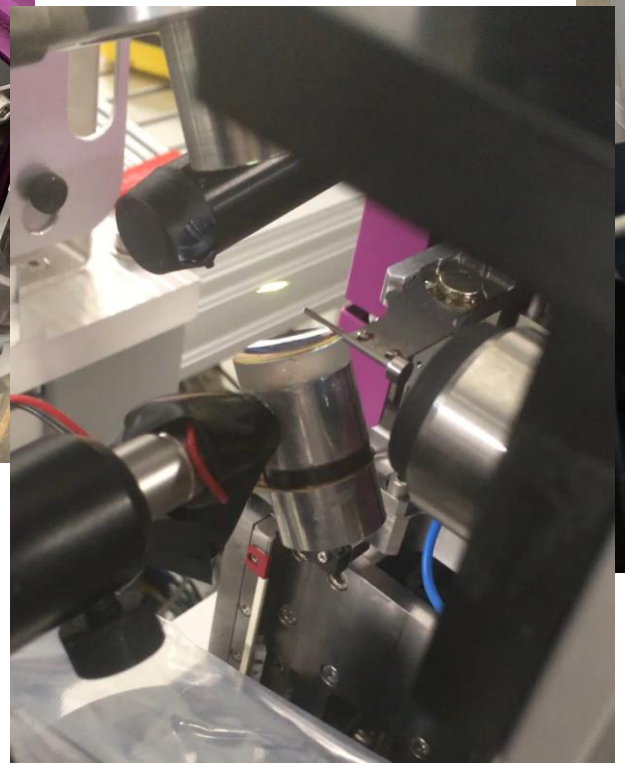


Movie of diffraction images collected with 70 us exposure time at ~700 Hz repetition rate

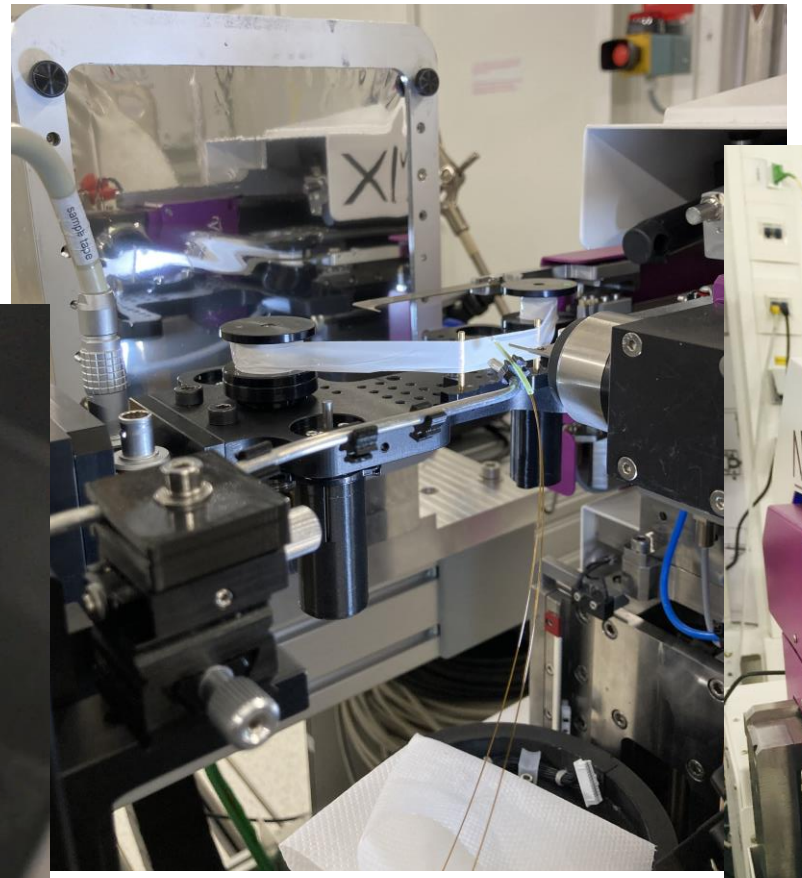
ID29 BEAMLINE- SAMPLE DELIVERY PORTFOLIO: FIXED TARGET



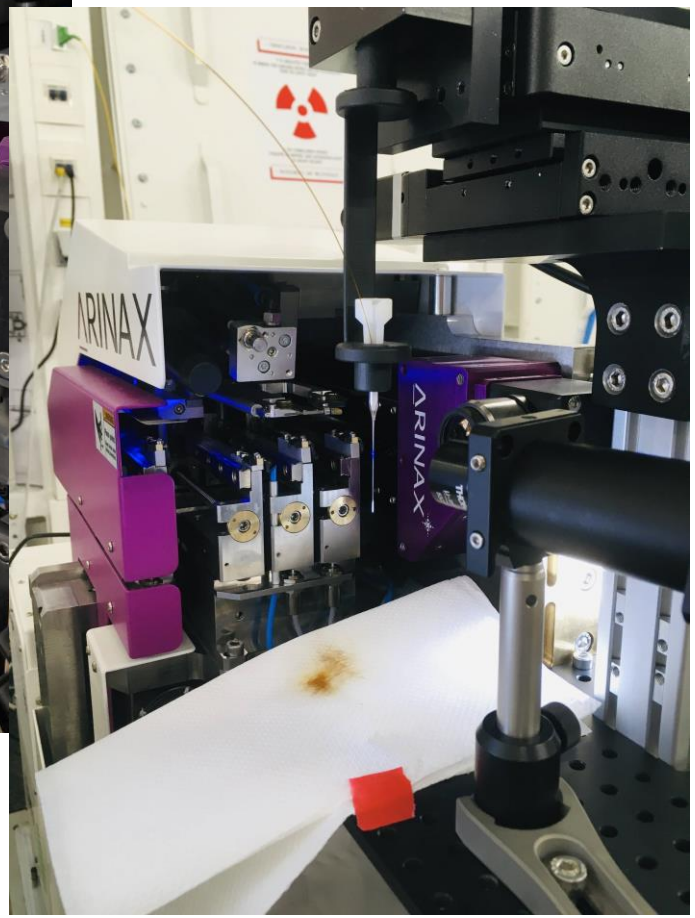
MPI-SOSchip



PSI Acoustic Levitator

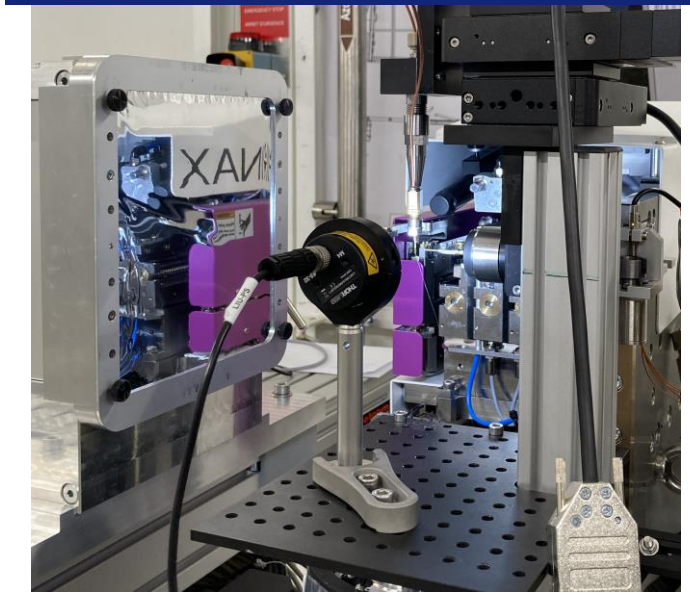


Tape-Drive
Lübeck University

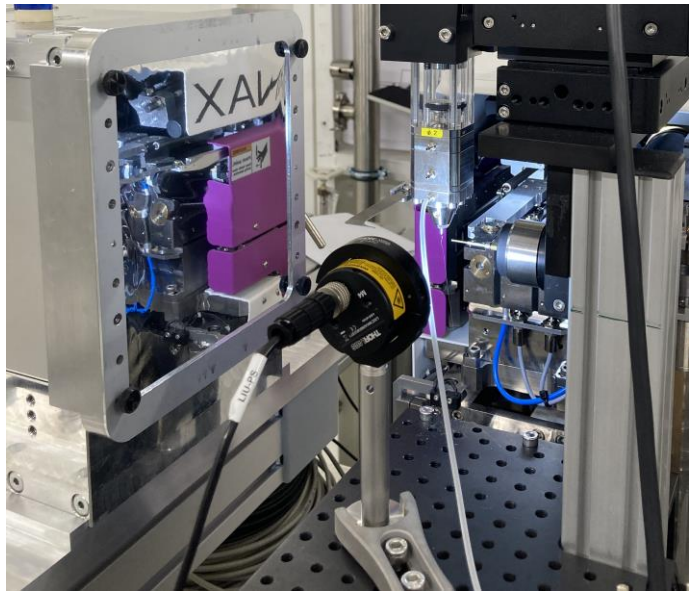


SerialX Gothenburg University

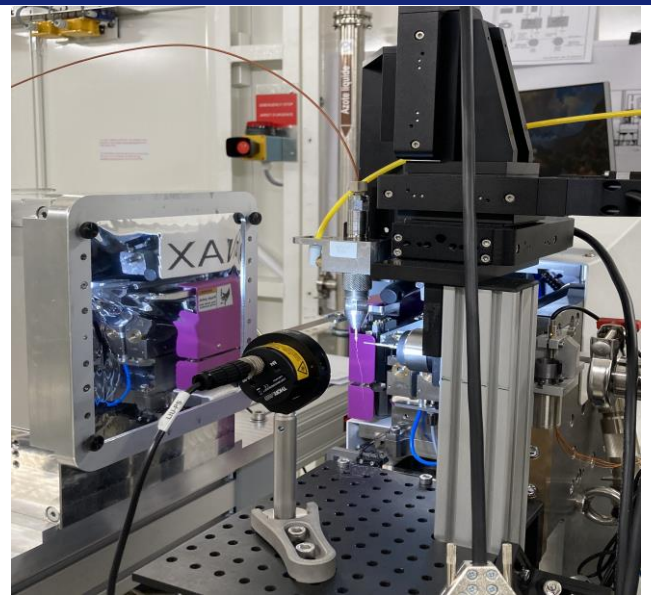
ID29 BEAMLINE- SAMPLE DELIVERY PORTFOLIO: HIGH-VISCOSITY INJECTORS



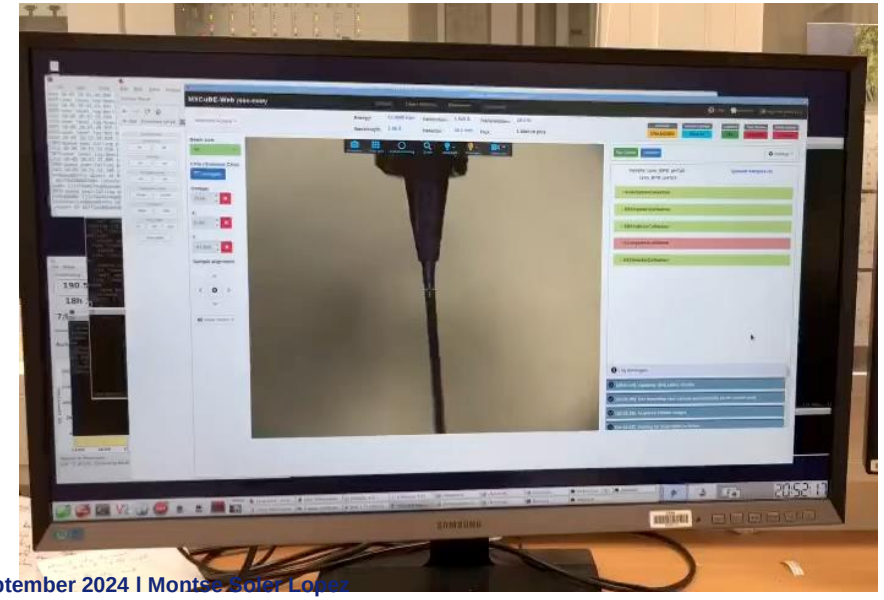
ASU injector



SACLA injector



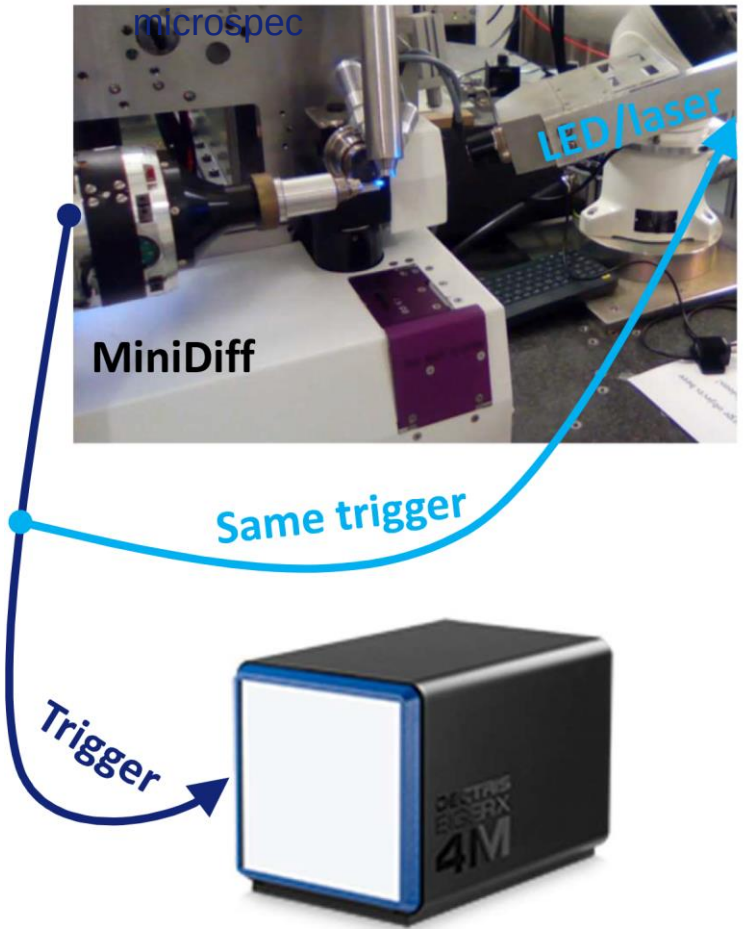
MPI injector



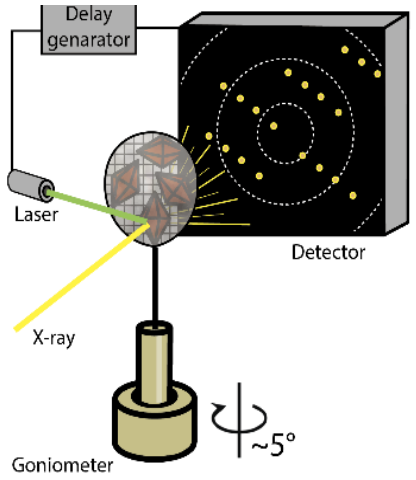
time-resolved serial oscillation crystallography (TR-SOX)

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

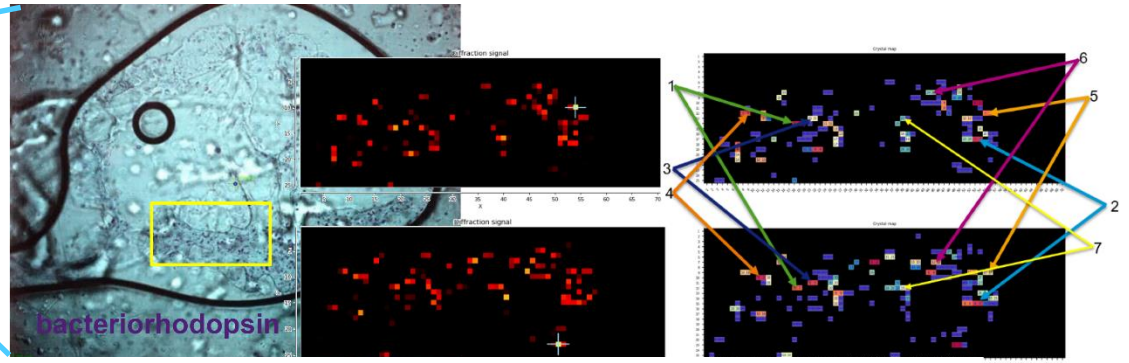
online UV-vis absorption microspec

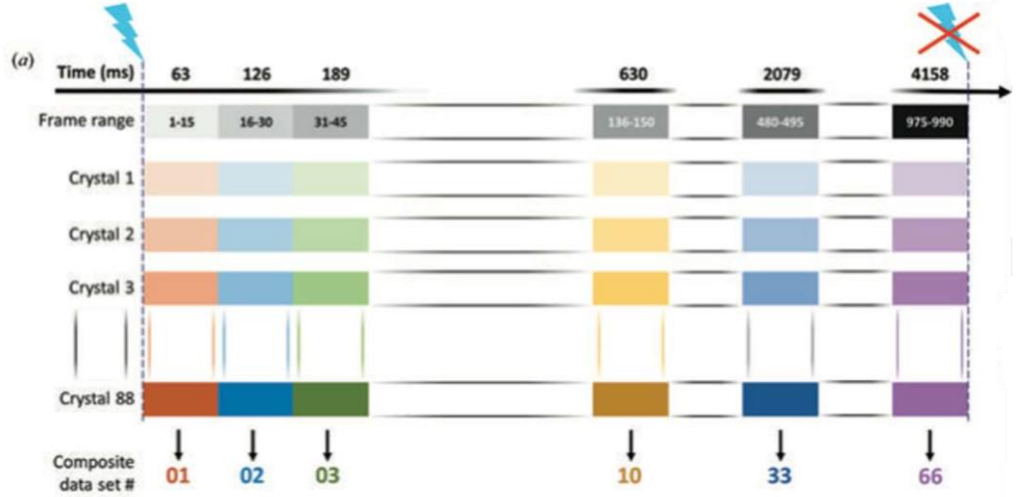


COC-sandwich on a 3D-printed single-hinge clamp



precise multi-crystal data collection using double mesh DOZORM2

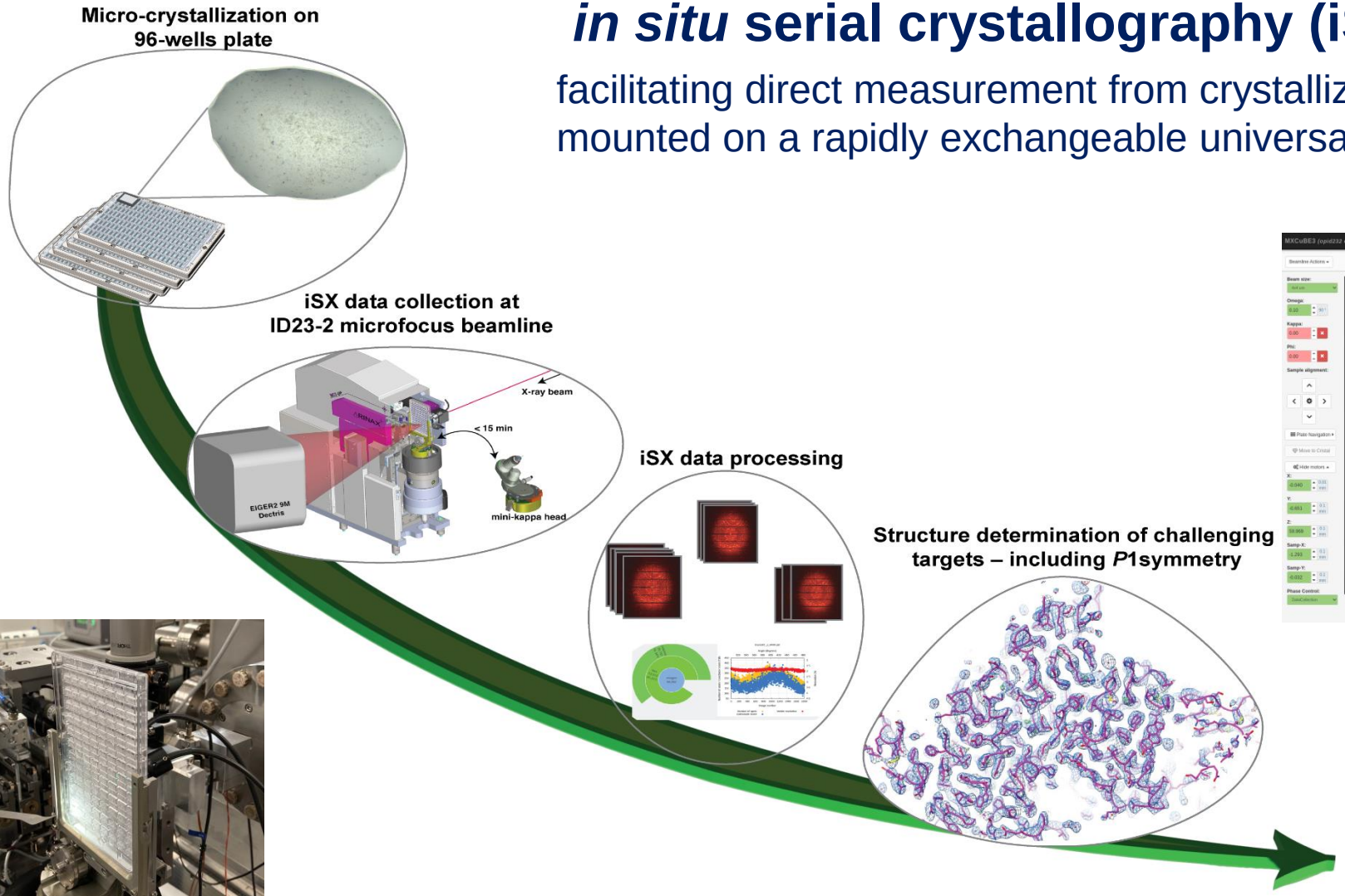


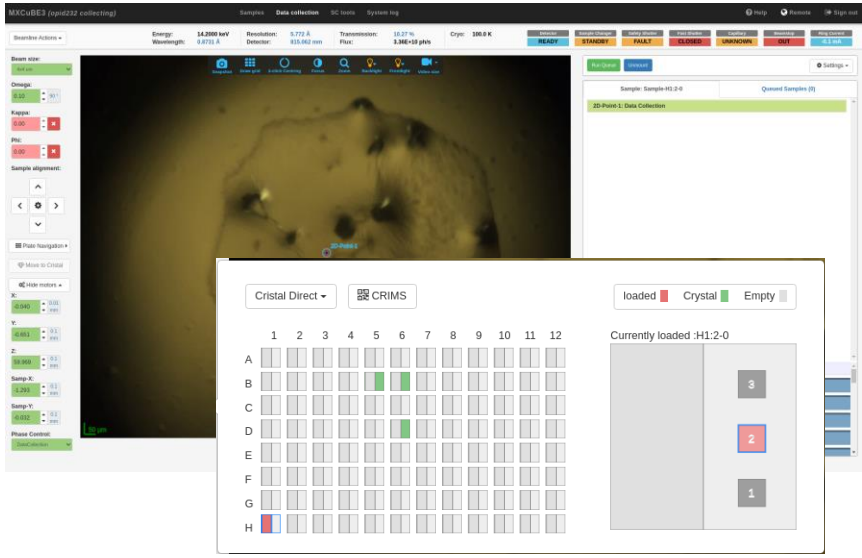


Aumonier et al. IUCrJ 2022

in situ serial crystallography (iSX)

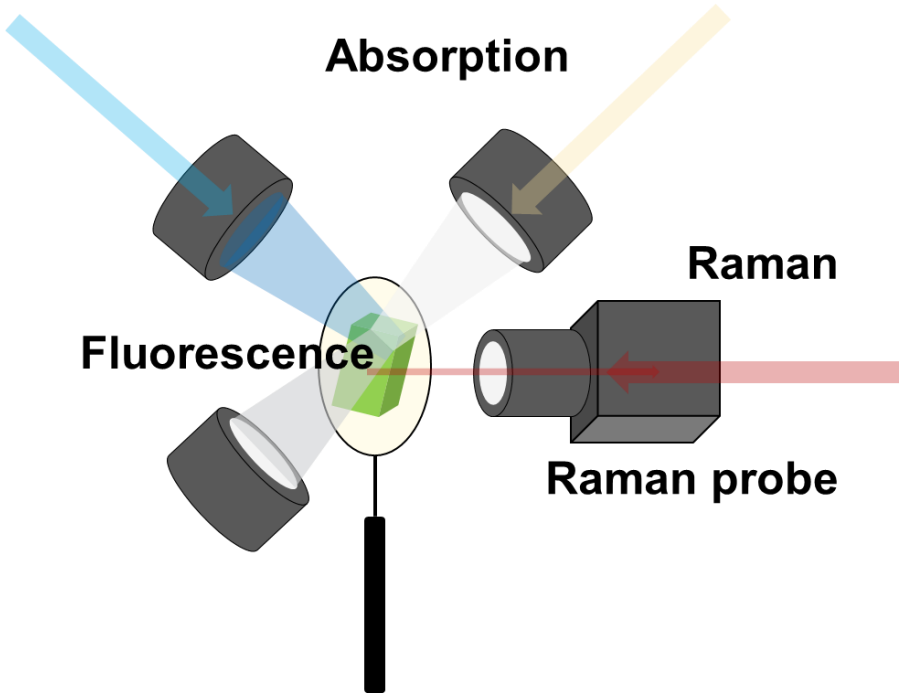
facilitating direct measurement from crystallization plates mounted on a rapidly exchangeable universal plate holder



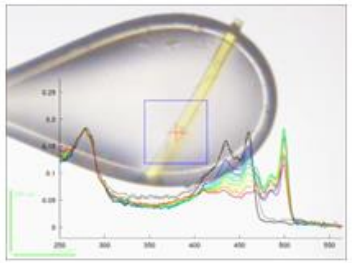


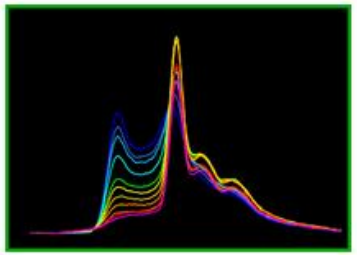
Foos N ,et al. IUCrJ. 2024

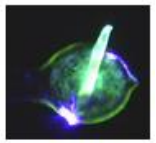
IN CRYSTALLO OPTICAL SPECTROSCOPY: THE icOS FACILITY

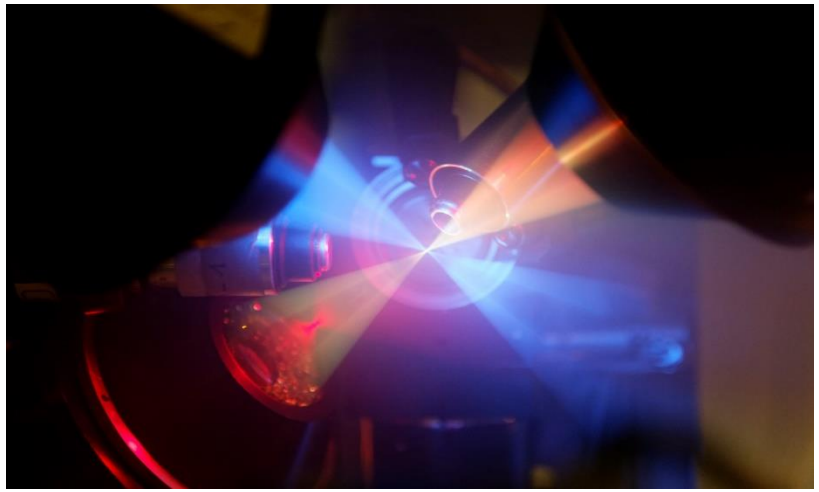


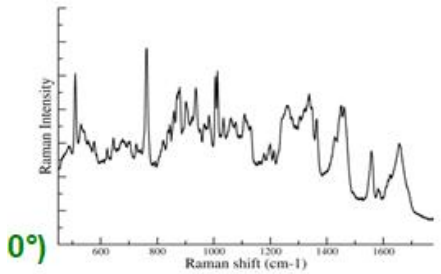
icOS





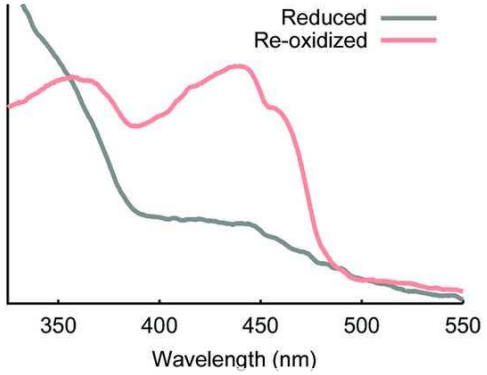






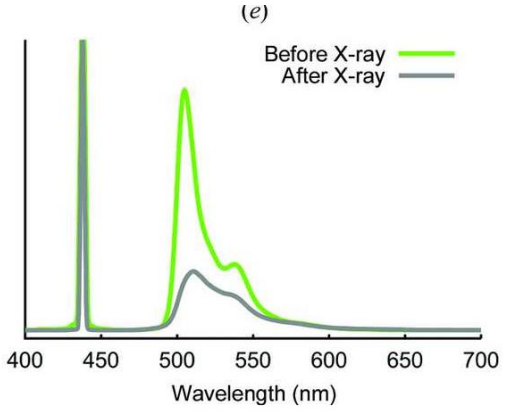
IN CRYSTALLO OPTICAL SPECTROSCOPY: THE icOS FACILITY

UV-Vis Absorption



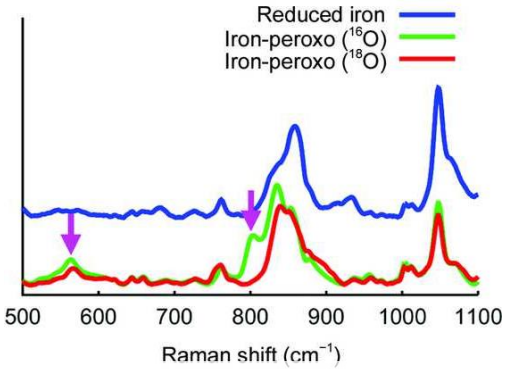
Functional state/
Radiation damage

Fluorescence



Radiation damage

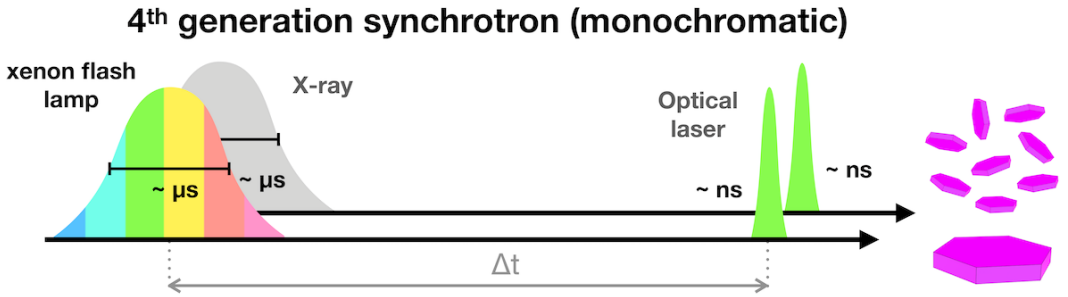
Raman



Kinetics MX/
Radiation damage

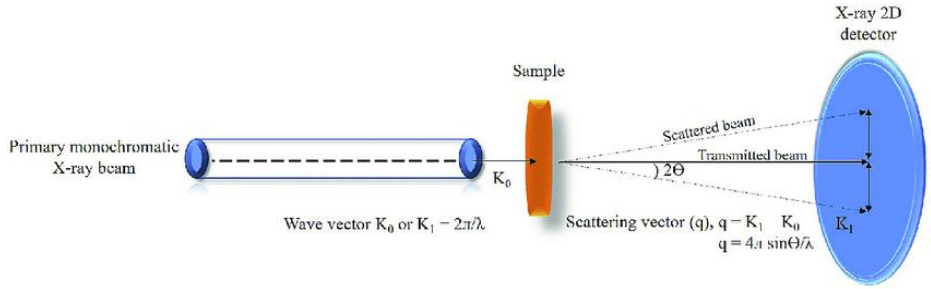
- Metal centres: Fe, Cu, Co
- Cofactor: flavins, NADPH, chlorophylls
- ***Photoactive proteins*** (flavins, retinal, biliverdin)
- ***Fluorescent proteins*** (cyan, red, near-infrared)

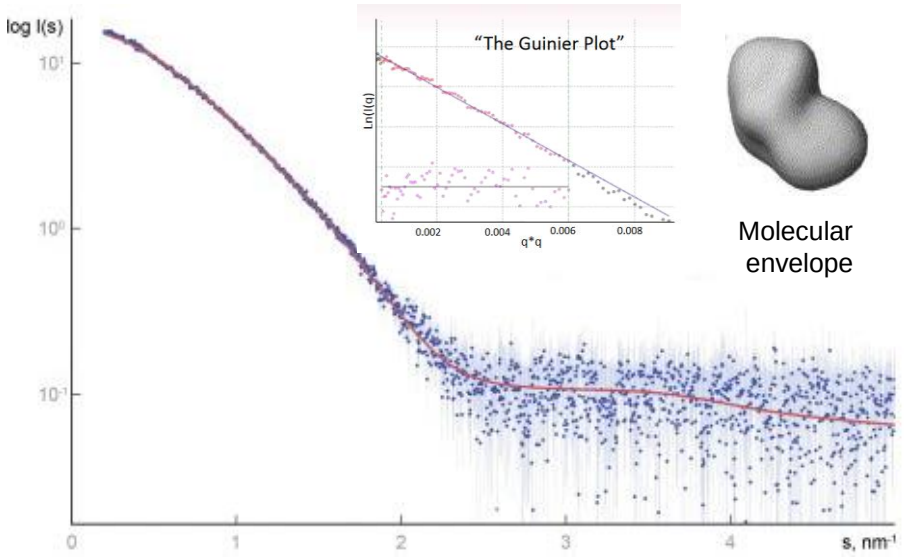
Time-resolved serial synchrotron (or millisecond) crystallography (TR-SSX/TR-SMX)



Engilberge et al. Acta Crystallogr D Struct Biol. 2024

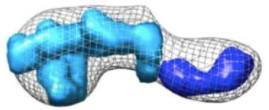
SAXS ‘measures’ the solution state of protein or biopolymer



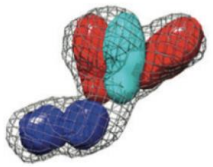


Molecular envelope

1. subunit organization



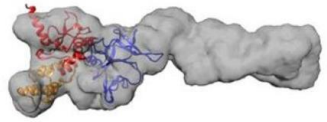
extracellular matrix proteins
(*Baldock Univ. Manchester*)



regulator bacterial cell division
(*Lewis Univ. Newcastle*)

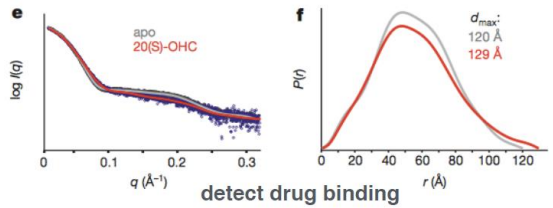
SAXS experiments

2. low-resolution shapes



protein antibiotic
(*Kleanthous Univ. Oxford*)

3. Monitor changes in conformational state

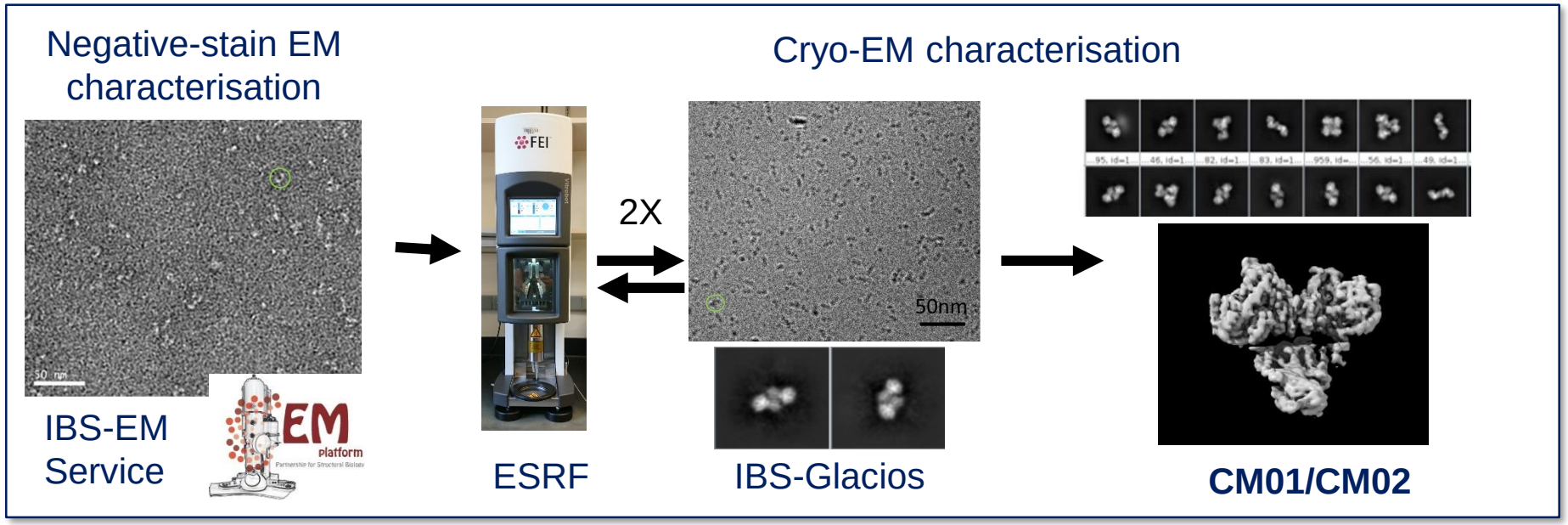


detect drug binding
(*Siebold Univ. Oxford*)

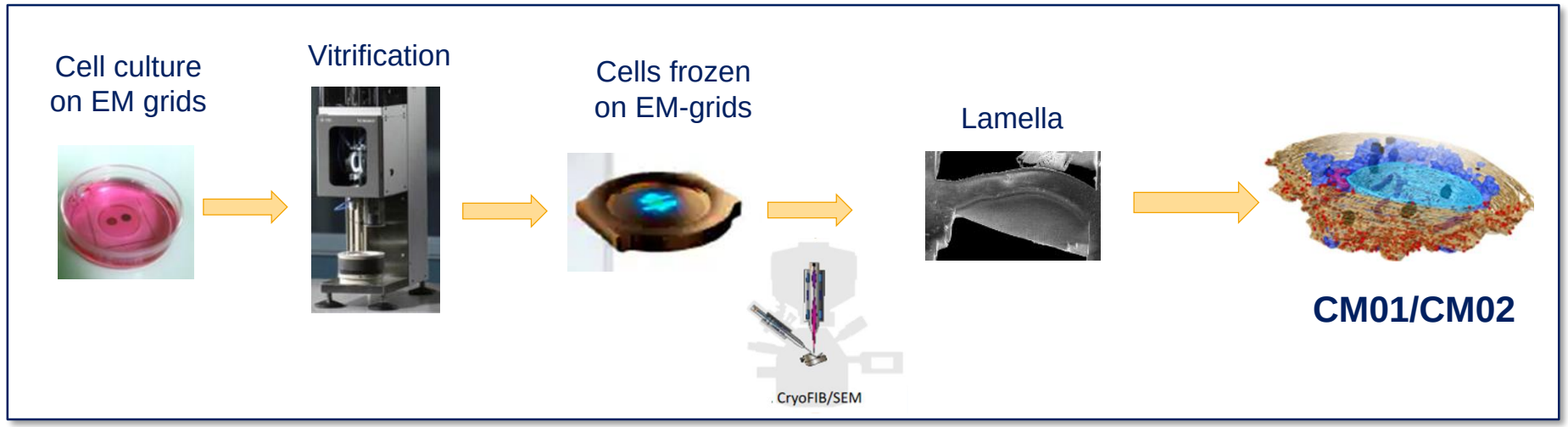
Hutin S, et al. Adv Exp Med Biol. 2024

STRUCTURAL BIOLOGY AT SYNCHROTRONS WITHOUT CRYSTALS – CRYO-ELECTRON MICROSCOPY (CRYO-EM)

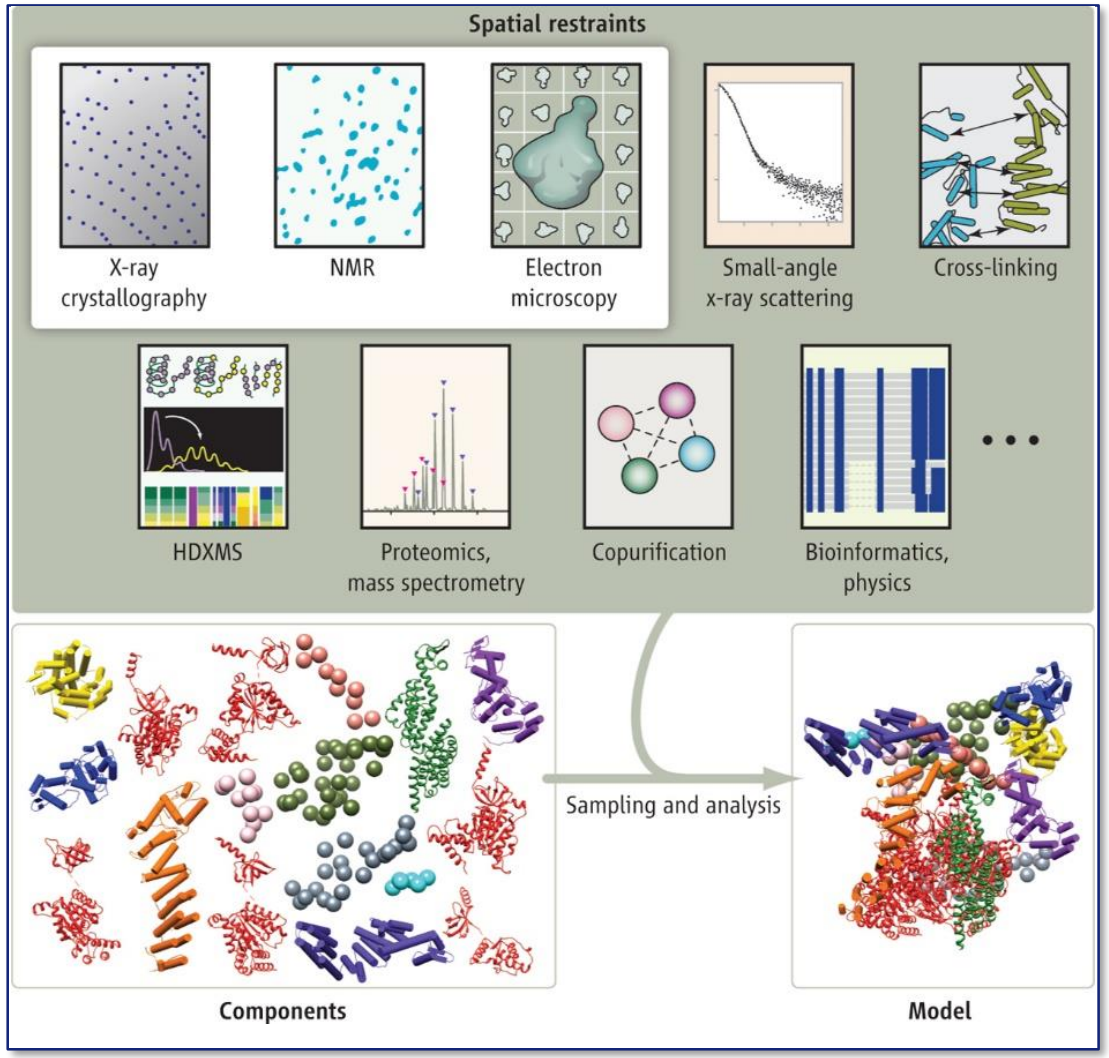
- From solution to EM structure (SOS) pipeline



- cryo-Electron Tomography (cryo-ET)

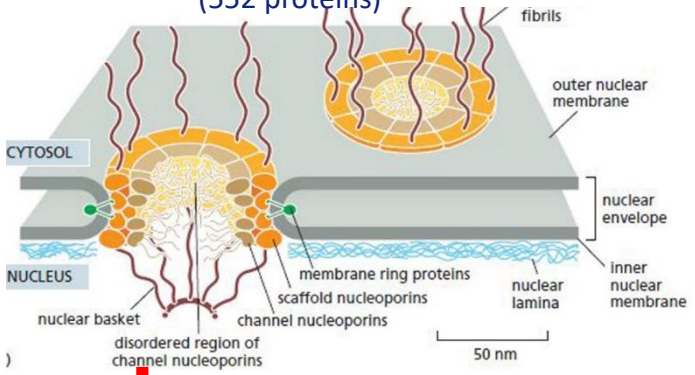


THE ROLE OF STRUCTURAL BIOLOGY IN THE NEW BIOLOGY: INTEGRATIVE STRUCTURE DETERMINATION



nuclear pore complex

(552 proteins)



before integrative biology

after integrative biology

Science Jun 2022 376, Issue 6598

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

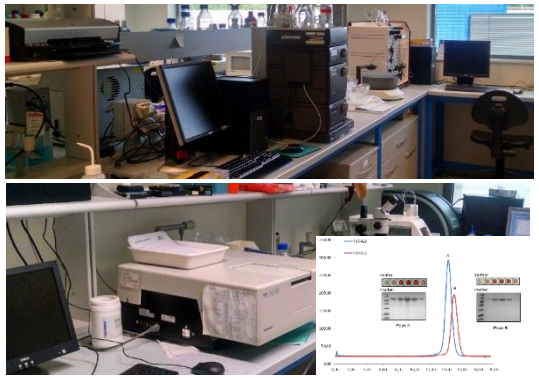
- MALS(SEC)
- AUC
- ITC
- SPR
- MST
- CD
- DLS
- Mass spectrometry
- Mass photometry
- spectroscopy

PROTEIN SUPPORT LABS

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

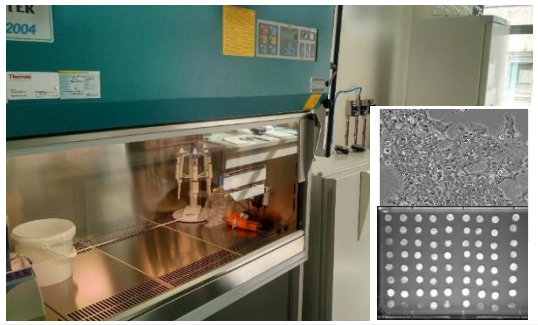
protein overexpression

Protein purification and biochemistry



Crystallisation & Cryocooling

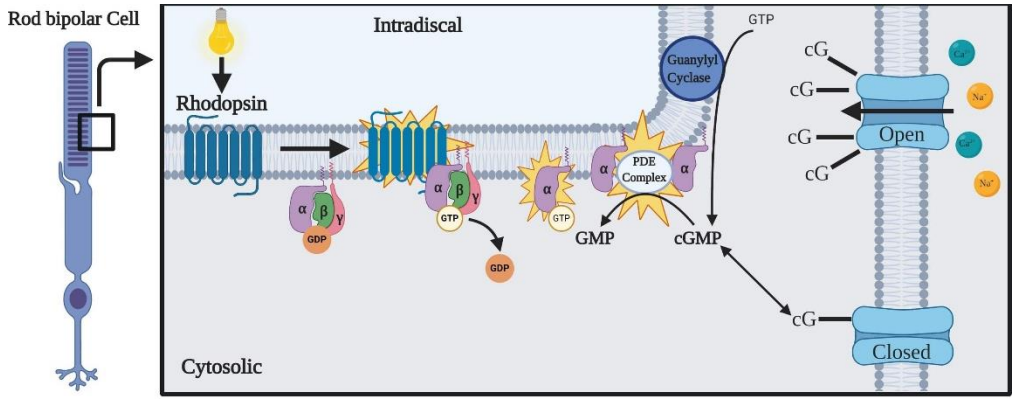
Cell culture (mammalian, yeast)

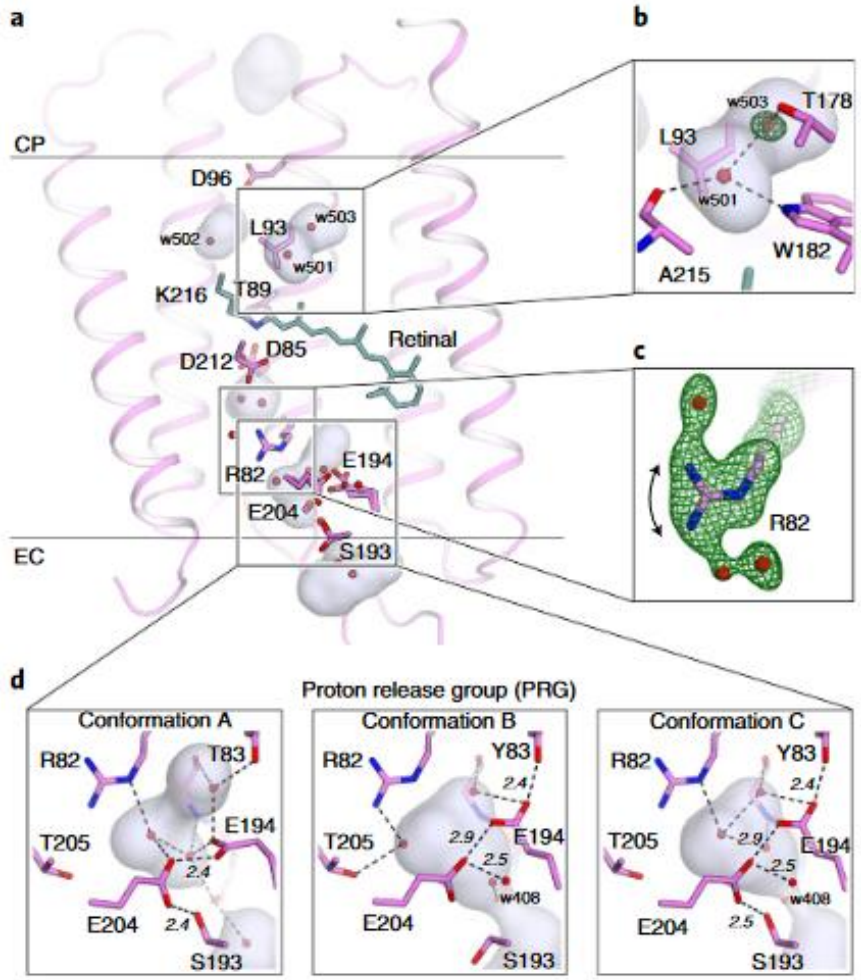


https://www.esrf.fr/home/UsersAndScience/support-and-infrastructure/support-labs/protein-support-labs.html

BIOTECHNOLOGY CHALLENGE: THE PHOTOCYCLE OF RHODOPSINS: LIGHT-SENSOR OF VISION

We have obtained atomic resolution structures of Bacteriorhodopsin photocycle intermediates, responsible for converting photons into chemical signals allowing to sense light





Melknikov, PhD 2017
Bukhdruker, PhD 2023

Borshchevskiy, V. et al. Nat Commun 2022
Podoliak, E. et al. Nat Commun 2024

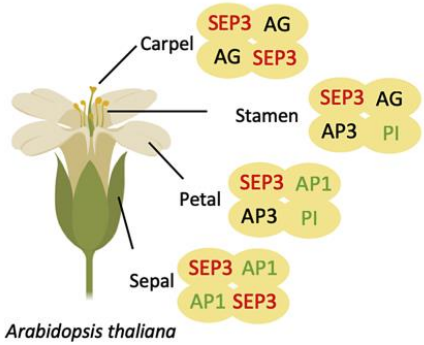
ENVIRONMENT CHALLENGE: UNDERSTANDING PLANT DEVELOPMENT

Gymnosperms

Flowering molecular mechanisms

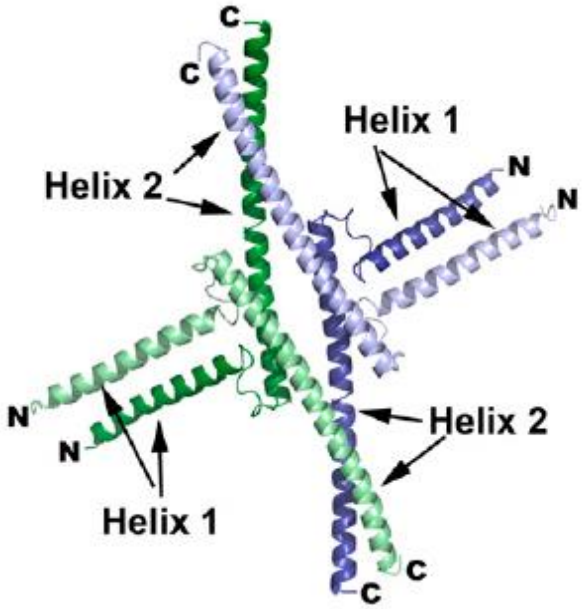
➢Root and fruit development

➢Flowering and floral organ development

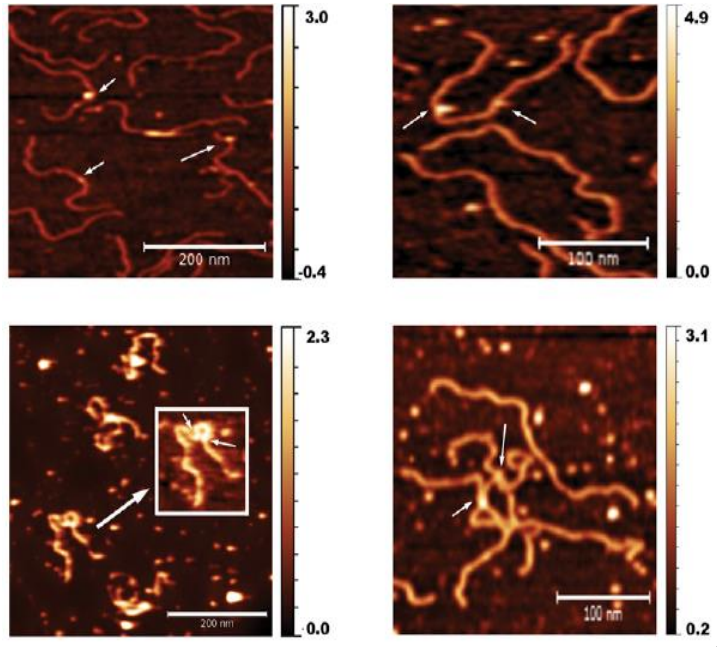


Angiosperms

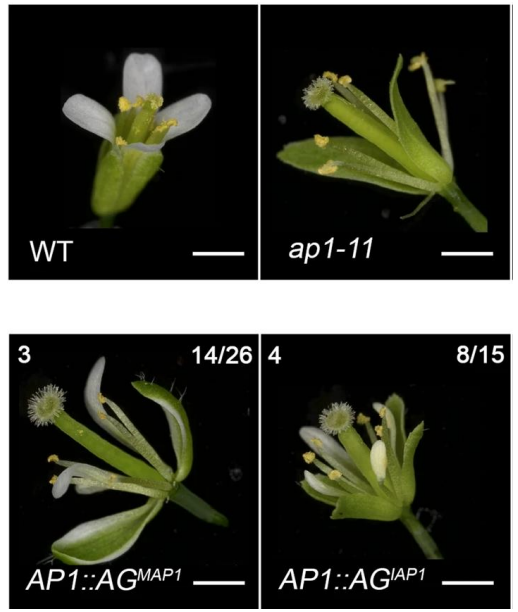
Structural biology of protein assembly



Biophysics of protein-chromatin assembly



In vivo analysis



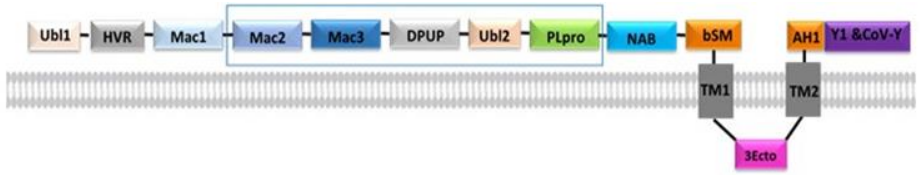
C. Zubieta
Laboratoire de physiologie cellulaire et végétale (LPCV)
CNRS / CEA / UGA / INRA

Puranik, PhD 2016
Mironova, PhD ongoing

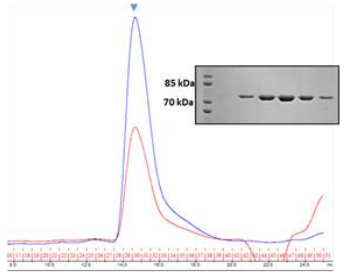
Hugouvieux et al. Plant Cell 2024; Lai et al. Nat Commun 2021; Puranik et al. Plant Cell 2014

PANDEMIC THREAT CHALLENGE: SARS-COV2

Structure determination of multi-domains by cryo-EM

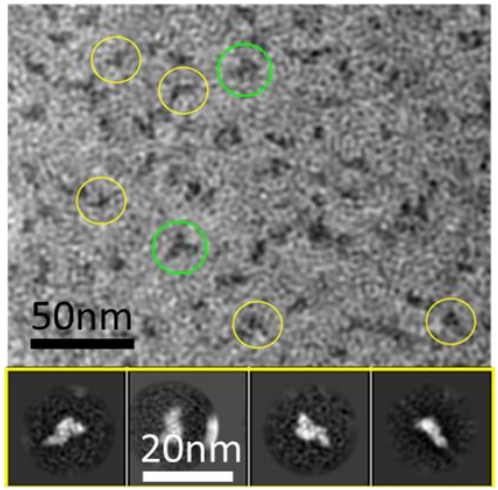


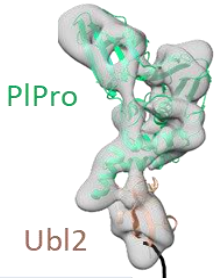
Nsp3-9 purified to homogeneity as a monomeric protein (76 kDa)

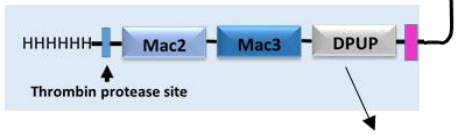


Data collected on Nsp3-9 in CM01(ESRF) using Phase plate

3D -reconstruction of Ubl2-Plpro (37kDa) to 8Å resolution

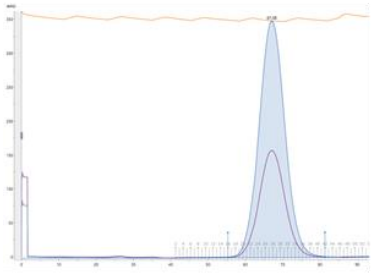


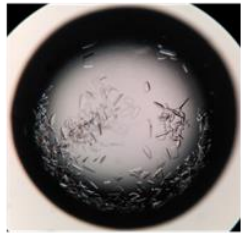




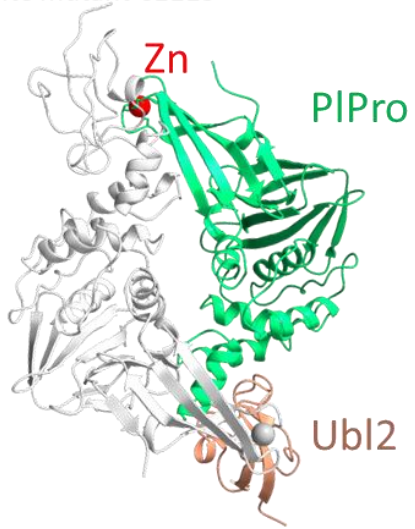
Not visible in the EM density

Structure determination of protease domain by MX





★ Active site mutant C111S



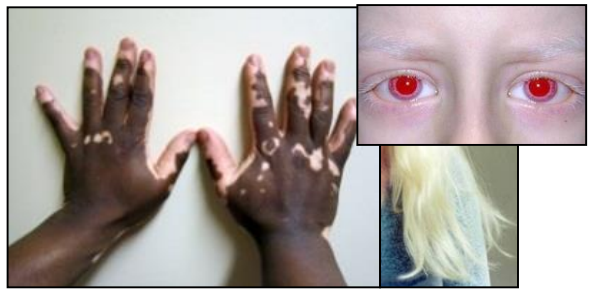
Crystal structure at 2.8Å

E. Kandiah

von Scholley, PhD ongoing

HEALTH CHALLENGE: SKIN BIOLOGY

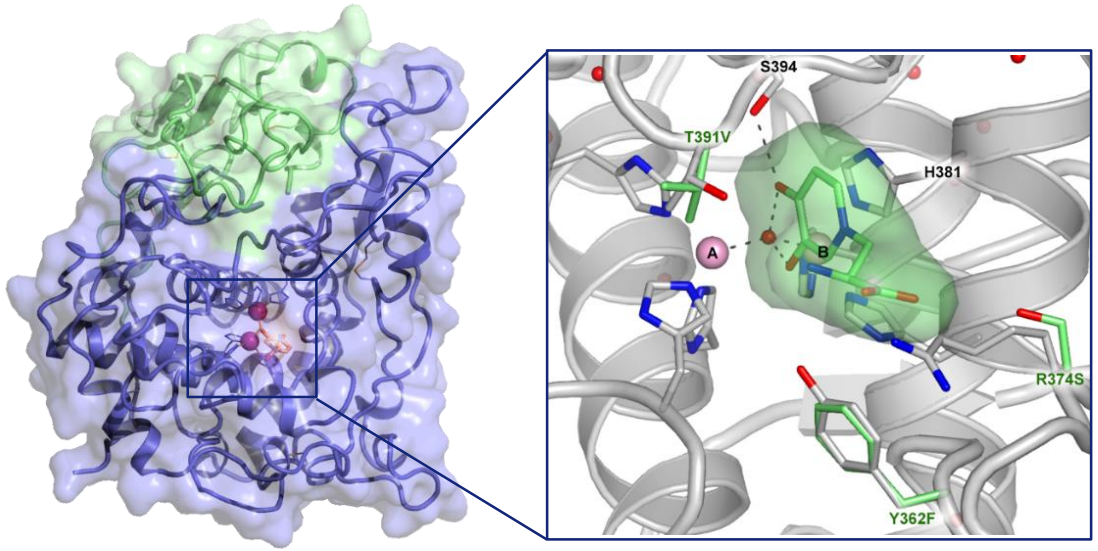
Melanin is the pigment responsible for the color of human skin, hair and eye
In humans, tyrosinases are metalloenzymes that catalyse the production of melanin from tyrosine



Mutations/variants ⇒

- Oculocutaneous albinism
- Pigmentation disorders
- Melanoma cancer

We solved the first structure of a human tyrosinase (TYRP1), with unexpected findings:



Crystal structure of TYRP1 protein in complex with a substrate at 2.3Å

Active site: two Zinc ions (purple) and a tyrosine substrate (green)

⇒

- TYRP1 contains zinc ions as metal cofactors, unlike tyrosinase
- TYRP1 does not show redox activity, unlike tyrosinase
- TYRP1 binds tyrosinase substrates

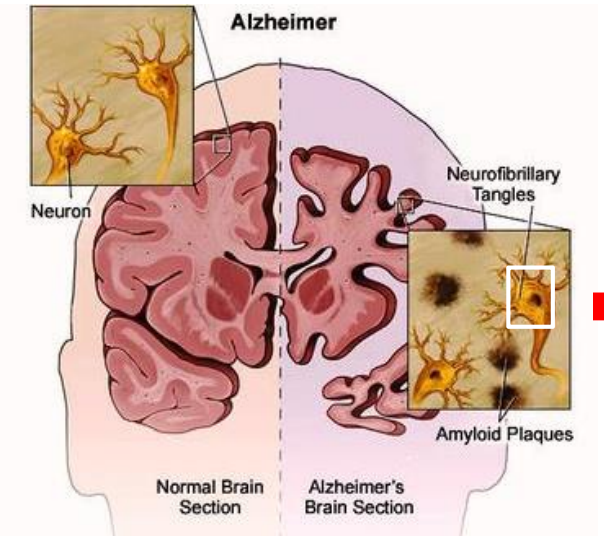
M. Soler-Lopez

Cosmethics Université Grenoble Alpes
InnovaXN
ETH zürich
immusmol

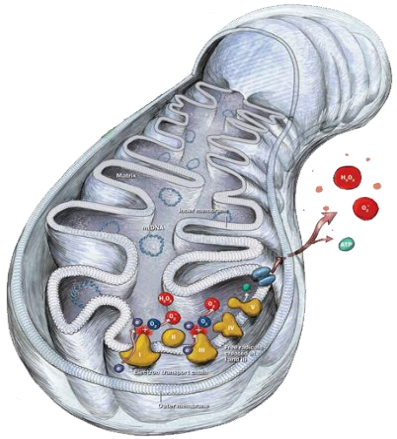
Lai, PhD 2017
Ng, PhD in October

Lai et al. PloS One 2016; Lai et al. Angew Chem Int Ed. 2017; Lai et al. Chemistry 2018; Lai et al. Int J Mol Sci. 2020; Faure et al. Chembiochem 2024

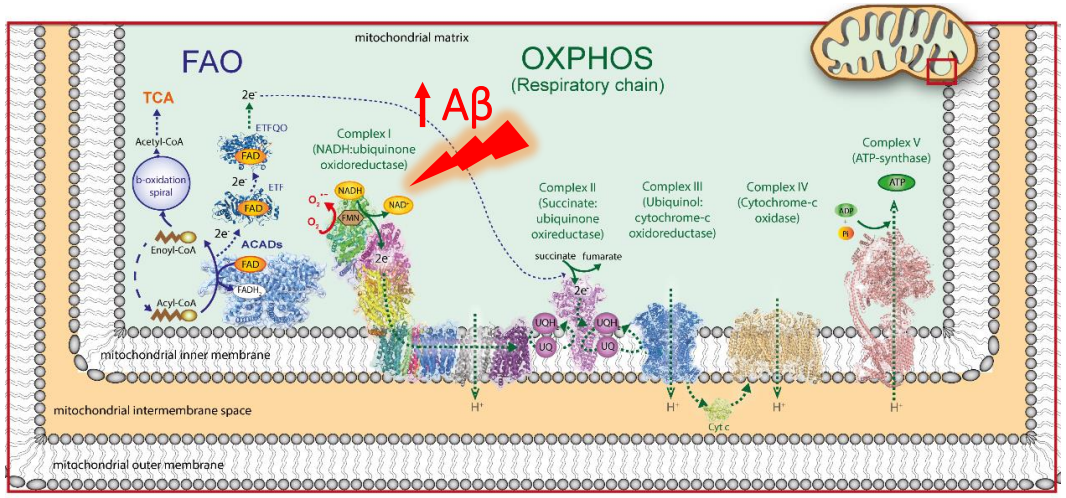
HEALTH CHALLENGE: INTEGRATIVE BIOLOGY TO INVESTIGATE THE ALZHEIMER'S DISEASE ROOT CAUSE



NEURONAL MITOCHONDRIA



IMPAIRED ENERGY METABOLIC PATHWAYS



There is **no cure or effective therapies** to treat the known hallmarks: amyloid-β plaques and tau fibrillary tangles. It is essential that **research focuses on the underlying molecular mechanisms** that initiate the disease, years before symptoms become apparent.

A popular hypothesis is that mitochondria, specifically the imbalance of energy pathways within mitochondria, are implicated in the root causes of AD.

However, due to a **lack of structural and functional data** on the mitochondrial protein complexes involved in mitochondrial dysfunction, their **roles are not fully understood**.

McGregor & Soler-Lopez, Current Opinion in Structural Biology 2023

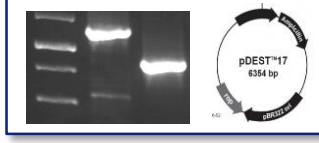
OUR INTEGRATIVE BIOLOGY APPROACH

PSB 19 platforms, 27 techniques, collaborations with IBS, EMBL, ILL

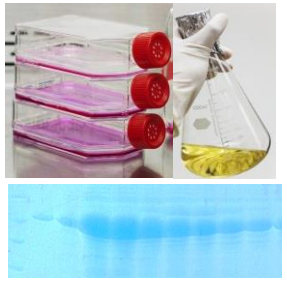
MX: ID23-1/2, ID30-A1/3, ID30B, ID29;
cryoEM CM01; SAXS BM29

Protein/DNA sample preparation

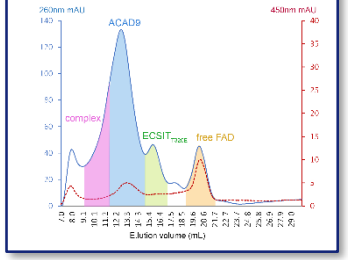
Gene cloning



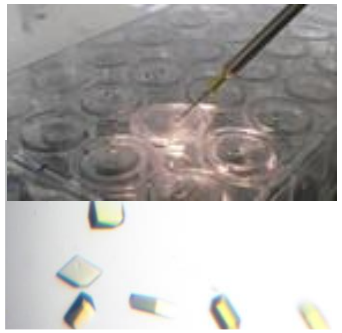
Protein production



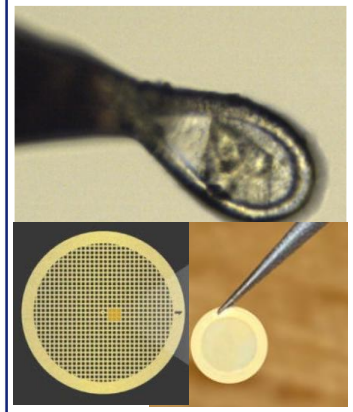
Complex purification



Crystallisation

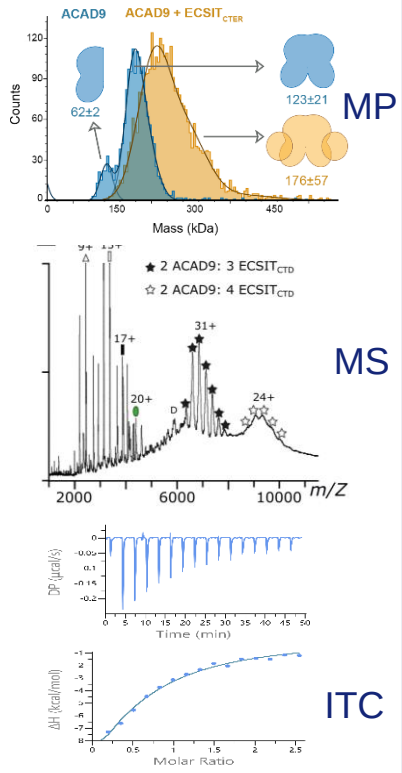


Cryocooling

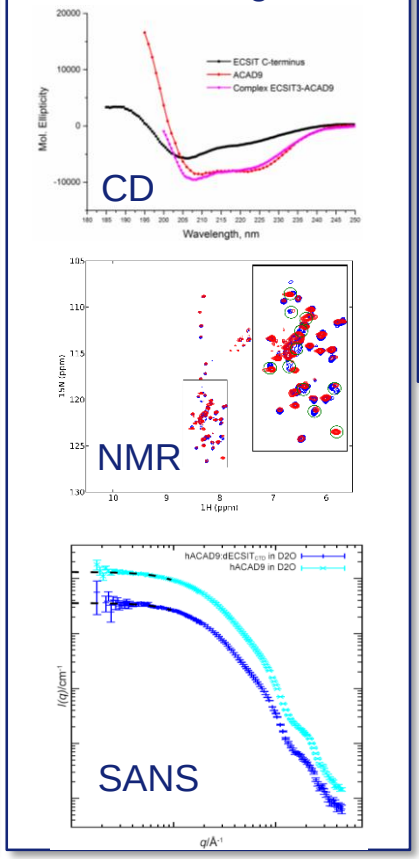


Biophysical characterization

Composition & Stoichiometry

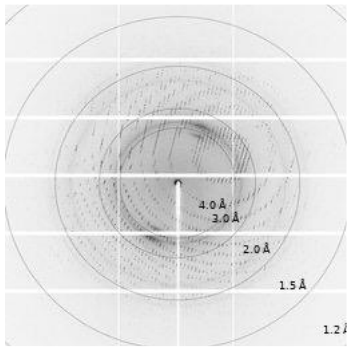


Folding

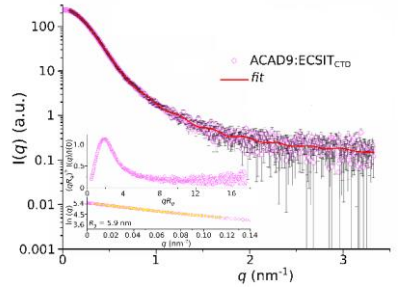


Structure determination of protein complexes

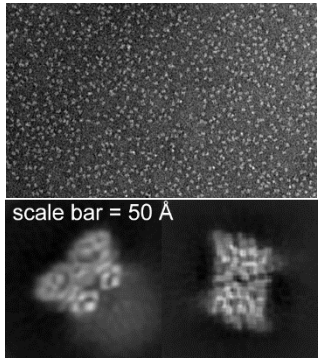
X-ray diffraction

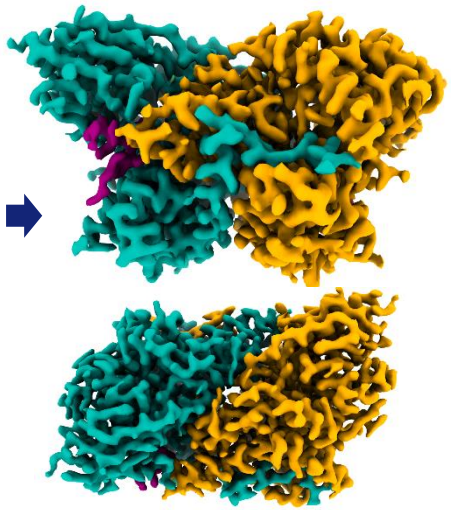


X-ray scattering



Electron microscopy



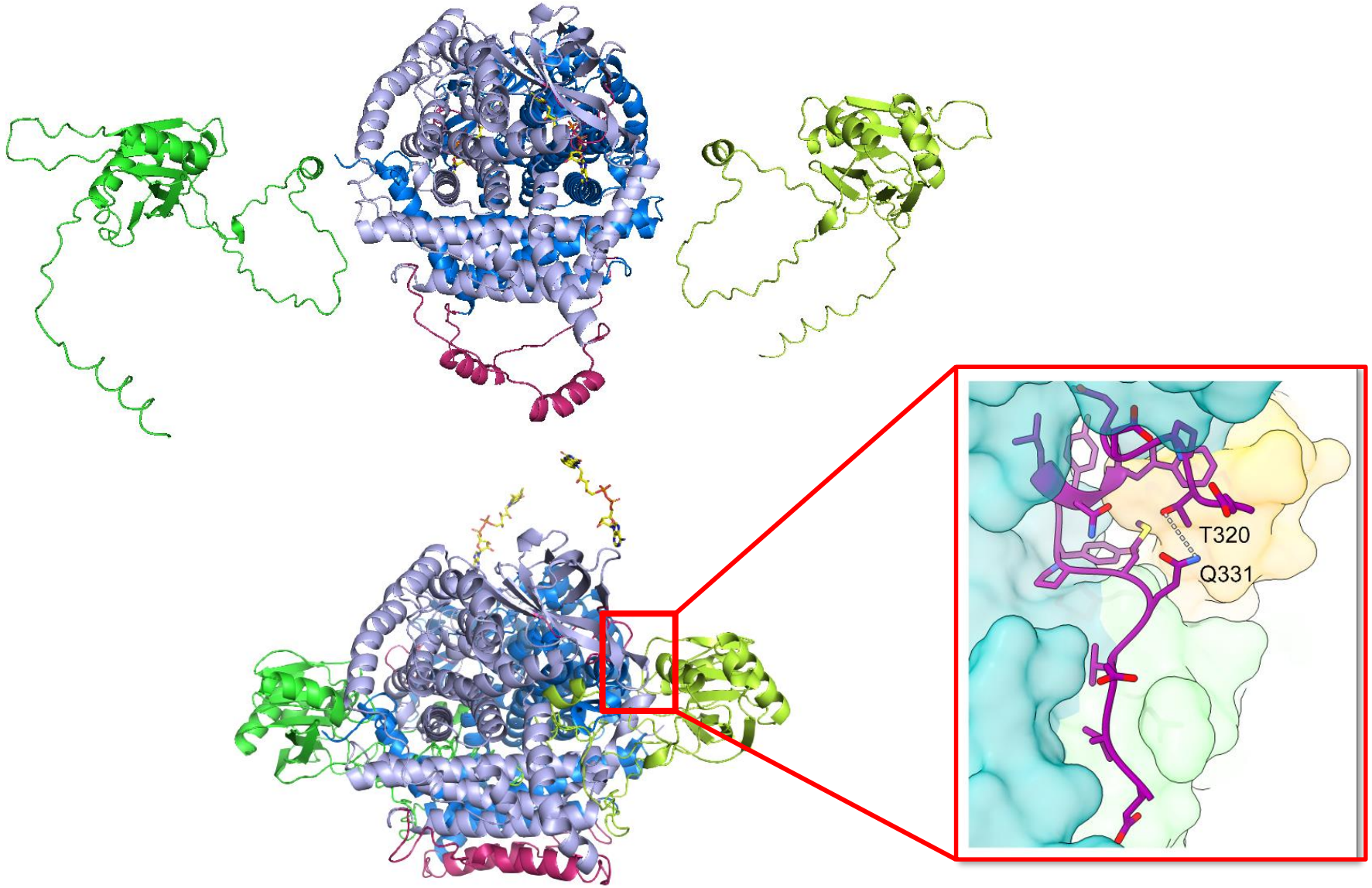


Giachin et al. Angewandte Chemistry Int Ed 2021; McGregor et al. Nature Commun 2023

FIRST RESULTS (I): THE MOLECULAR MECHANISMS OF PROTEIN COMPLEXES INVOLVED IN BIOENERGETICS

Fatty acid metabolism
~~*Respiration activation*~~

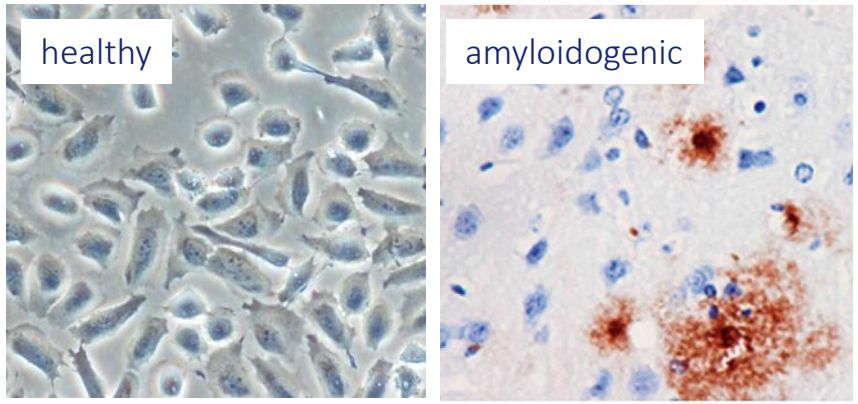
~~*Fatty acid metabolism*~~
Respiration activation



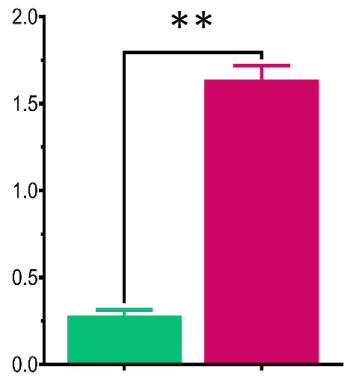
McGregor & Soler-Lopez. Current Opinion in Structural Biology 2023; McGregor et al. Nature Commun 2023

FIRST RESULTS (II): CELLULAR TOXICITY OF AMYLOID AGGREGATES IN ALZHEIMER'S DISEASE PROGRESSION

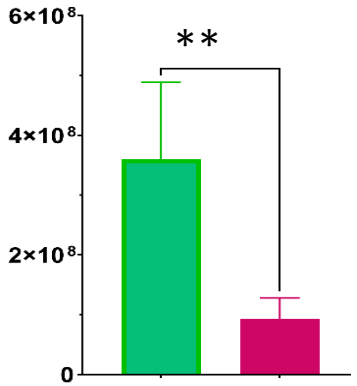
Neuronal cells



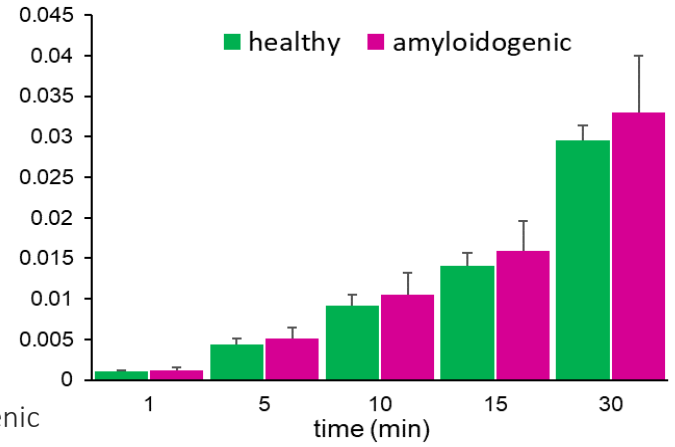
Amyloids in mitochondria



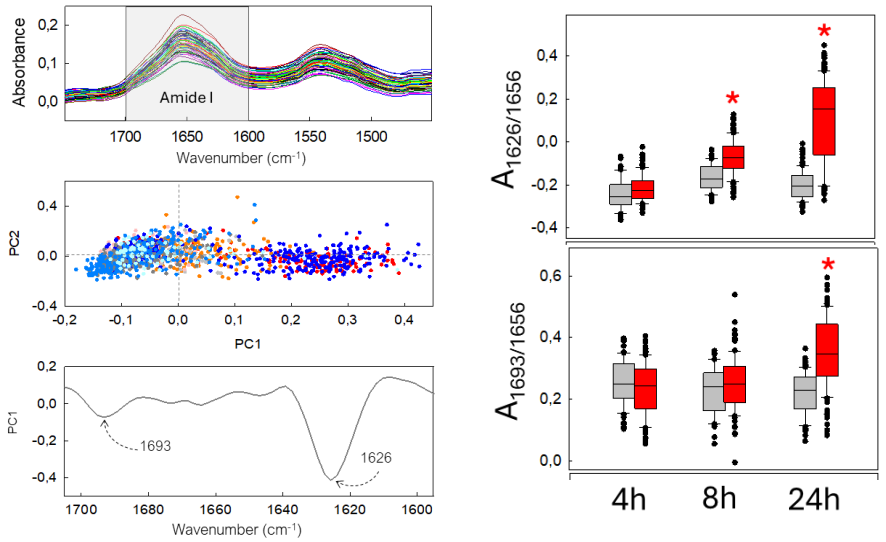
Phosphorylation



Energy production



Secondary structure of amyloids over time



µFTIR (MIRAS beamline, ALBA)

M. Soler-Lopez

Bouverot, PhD 2019
Magri, PhD ongoing
Boukherrouba, PhD ongoing

McGregor et al. Nature Commun 2023; unpublished data

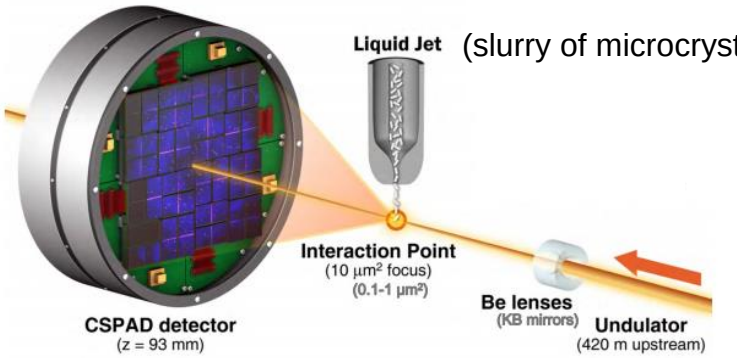
THANK YOU!

Partnership For Structural Biology

SERIAL FEMTOSECOND CRYSTALLOGRAPHY @ X-FELS

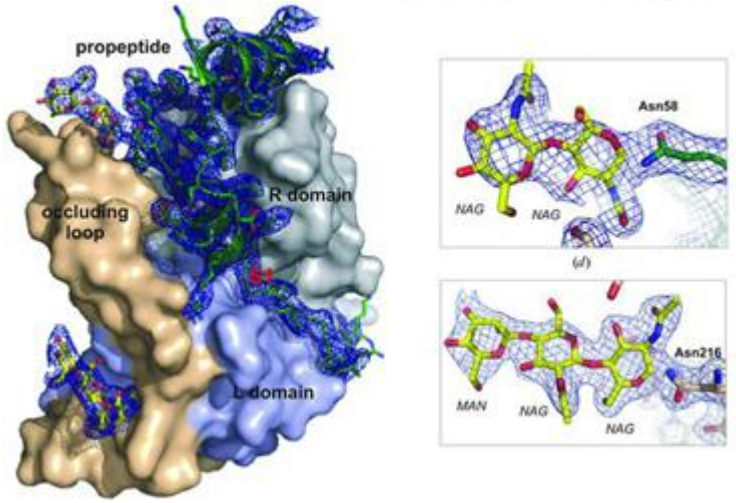
X-ray free-electron lasers (XFELs) generate coherent, ultra-brilliant, tunable laser pulses of very short duration in the X-ray regime the peak brilliance is 10 orders of magnitude higher than that currently achievable at synchrotrons

High resolution serial femtosecond crystallography



(slurry of microcrystals)

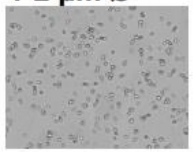
Cornell-SLAC Pixel Array Detector



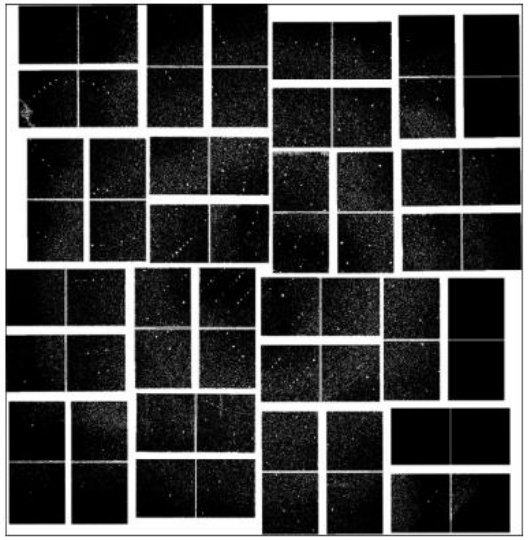
Redecke et al. (2013) Natively Inhibited Trypanosoma brucei Cathepsin B Structure Determined by Using an X-ray Laser. *Science* **339**, 227-30.

High resolution femtosecond diffraction of micron-sized lysozyme crystals

Lysozyme crystals
1-2 μm Ø



40 fs pulse
10 μm² focus
Transmission 15%
0.6 mJ/sample
33 MGy/pulse
9.4 keV , λ =1.32 A
Resolution 1.9 Å



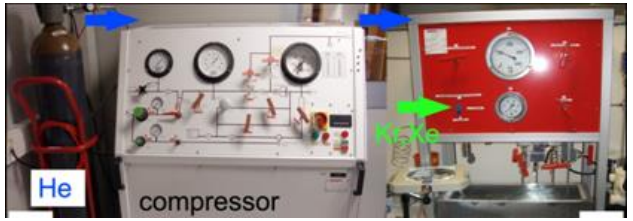
I. Schlichting, ESRF Users' Meeting, February 2014.

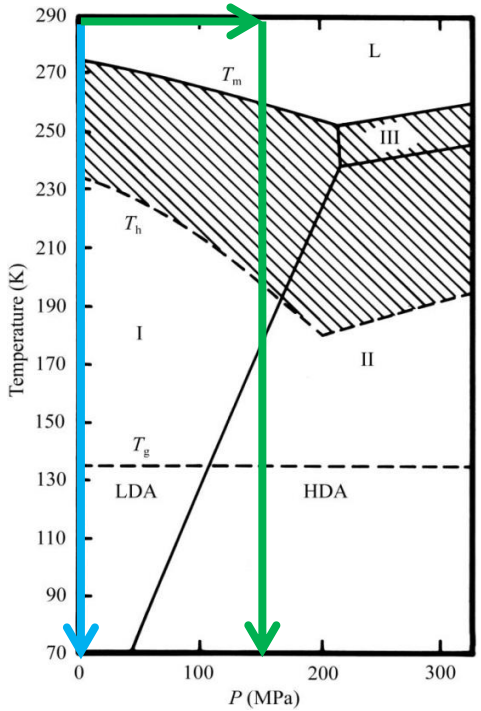
- Crystals pass through beam in random orientations. Single 'still' image from each crystal. (Tens of) 1000s of images combined to produce a complete data set

HIGH PRESSURE LABORATORY FOR CRYOCOOLING BIOLOGICAL MACROMOLECULES (HPMXL)

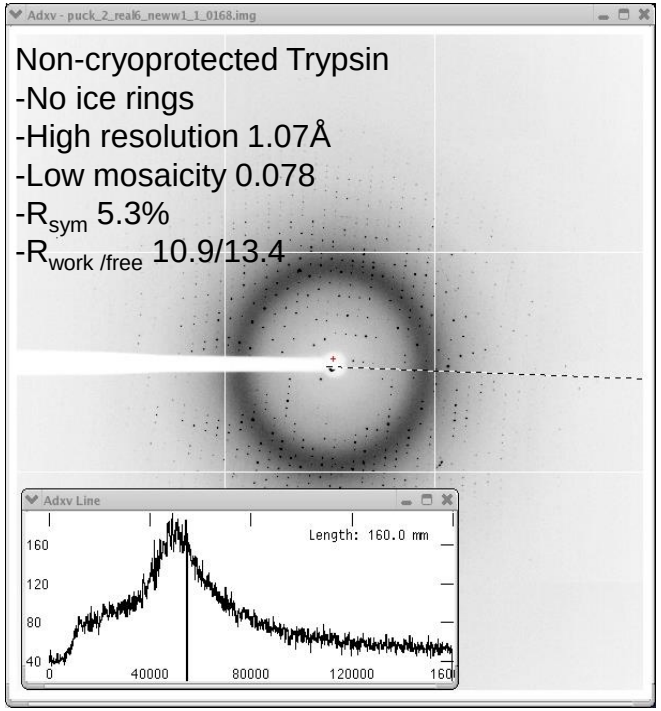
HPMXL

pressure derivatisation with O_2, Xe, Kr, …





- Cool crystals without cryoprotectant
- Improve diffraction quality (lower mosaicity)
- Where do ligands bind?
- Where is O_2/NO/… entering/leaving



van der Linden et al. (2014) J. Appl. Cryst. 47:584-592