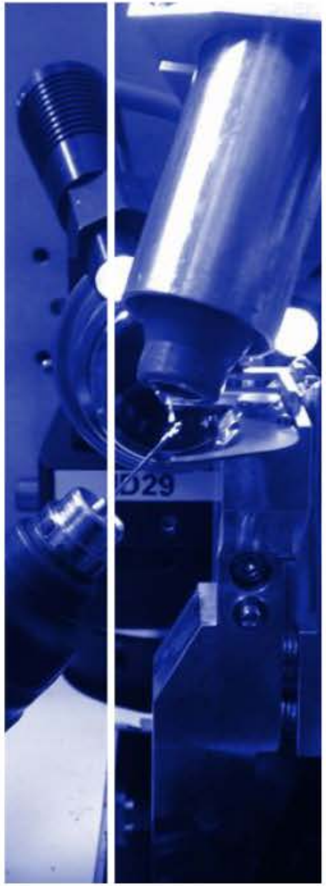


X-ray for Glyco structural biology

(Serial Crystallography at ID29)

daniele.de_sanctis@esrf.fr

OUTLINE

- Introduction
  - Traditional MX
- The opportunity provided by ESRF-EBS
  - Serial Crystallography
  - The new ID29
  - Possible applications

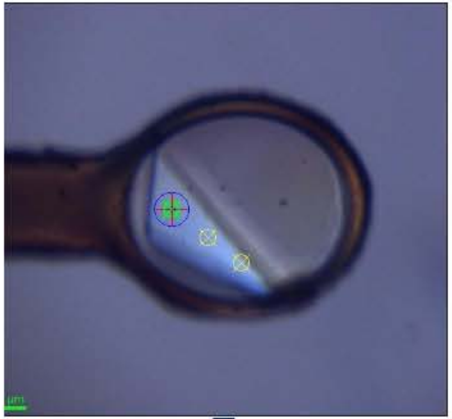
WHAT IS MACROMOLECULAR (CRYO)-CRYSTALLOGRAPHY?

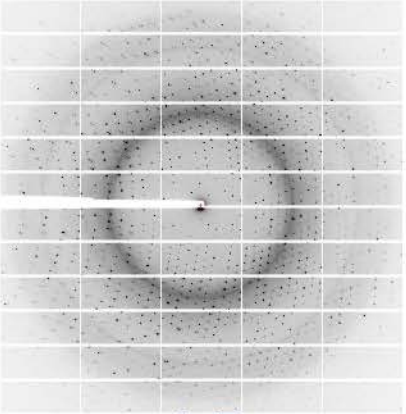
Crystals are cooled at 100 K

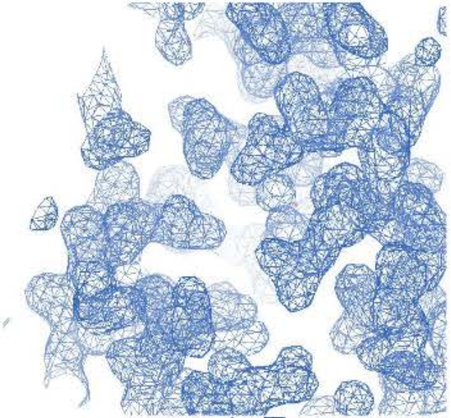
Diffraction intensities are recorded by rocking the crystal

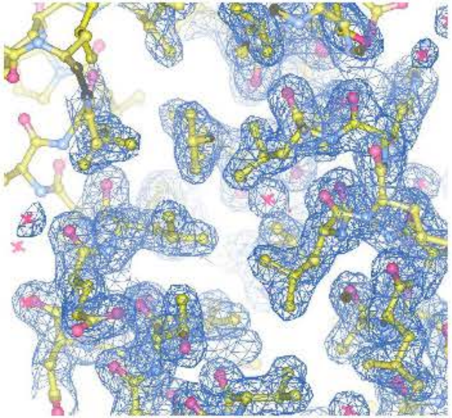
Electron density map is calculated

Atomic model is built









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

$$\rho(\mathbf{x}) = \mathcal{F}(\mathbf{F}(\mathbf{k})) = \int \mathbf{F}(\mathbf{k}) e^{-2\pi i \mathbf{k}\cdot\mathbf{x}}$$

EVOLUTION OF MX

- Many developments have contributed to the enhance the success of MX
  - Crystals
    - Protein production
    - Crystallogenesis (robots, nano-dispenser)
    - Cryocooling
  - Beamlines
    - Automation
    - Robotics
    - Detectors technology
    - Control software

BEAMLINE HARDWARE

Detectors
Faster detection
No-readout noise
Negligible readout time

Diffractometers
Motor accuracy and speed
Multi-axis synchronous movements

Robots
Automatic sample mounting
High capacity and autonomy

- These developments have allowed to
  - Exploit microbeam
    - Collect data from microcrystals, optimise signal-to-noise
    - Obtain high(er) quality data
  - Increase the throughput
    - Determine more structures
    - Enable fragment screening strategies for drug design
    - Develop beamline completely automated (Massif1)
  - Provide better structural information, more complete, better contributing to elucidate the biological mechanisms

SOME HIGHLIGHTS

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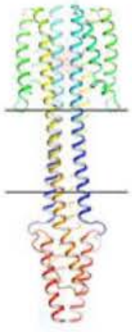

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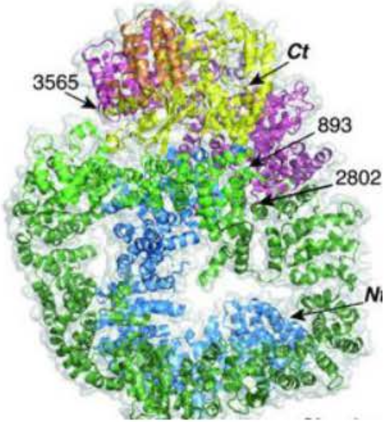

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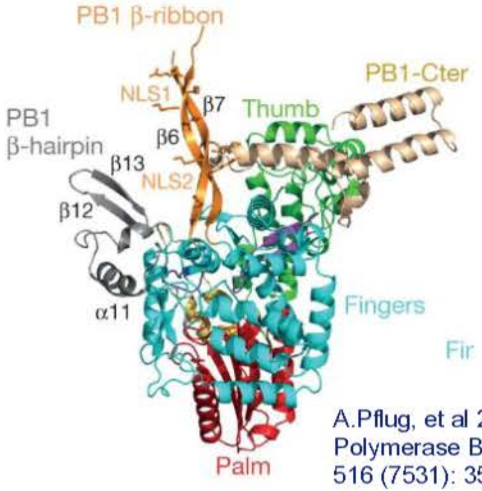

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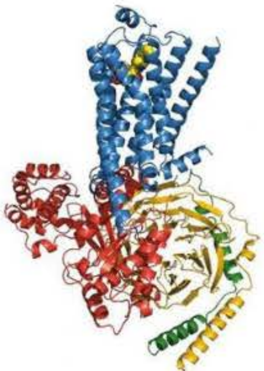
SOME HIGHLIGHTS



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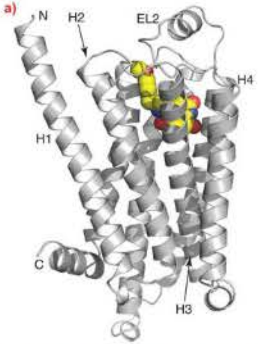

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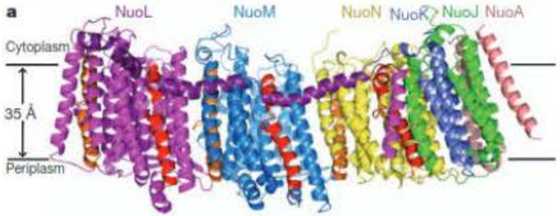
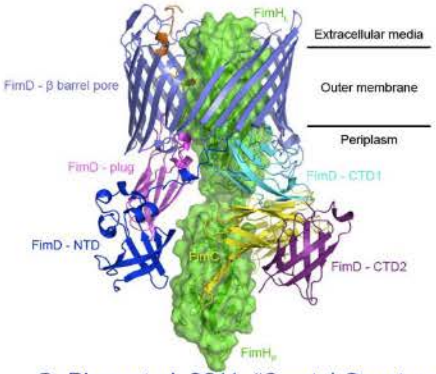


R.G. Efremov and L.A. Sazanov, 2011 Nature, 476, 414–420 "Structure of the Membrane Domain of Respiratory Complex I." Nature 476 (7361): 414–20.



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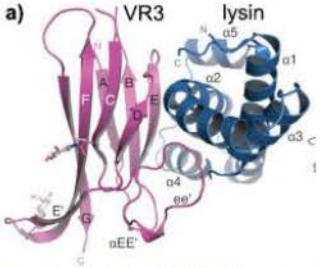

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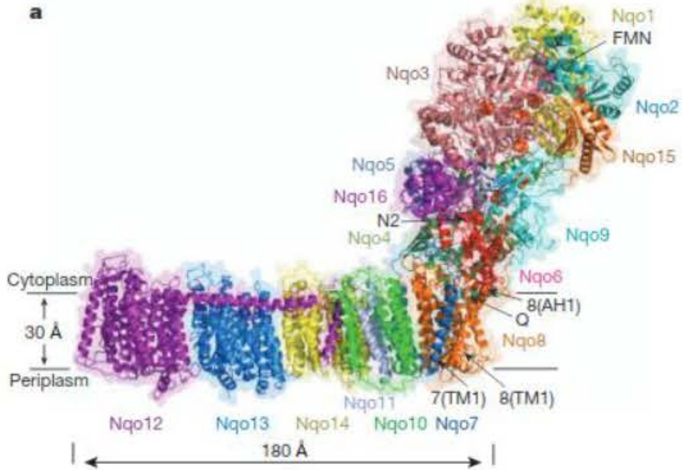

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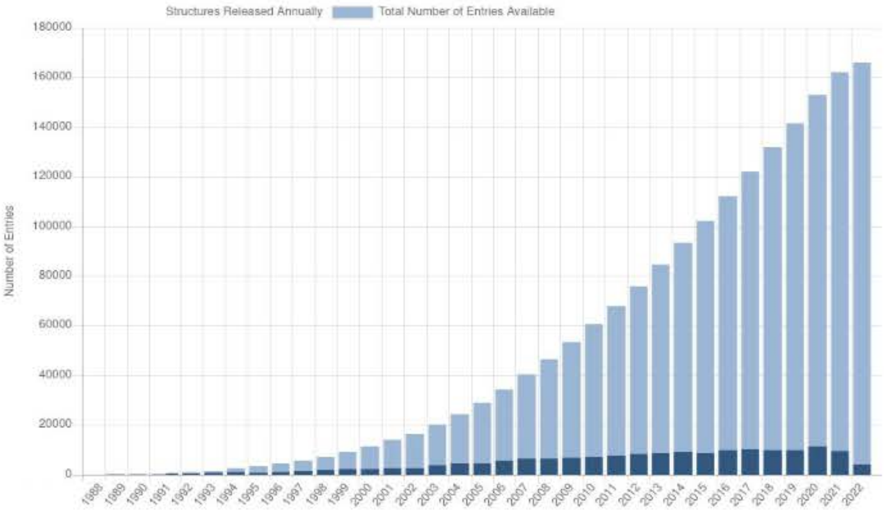
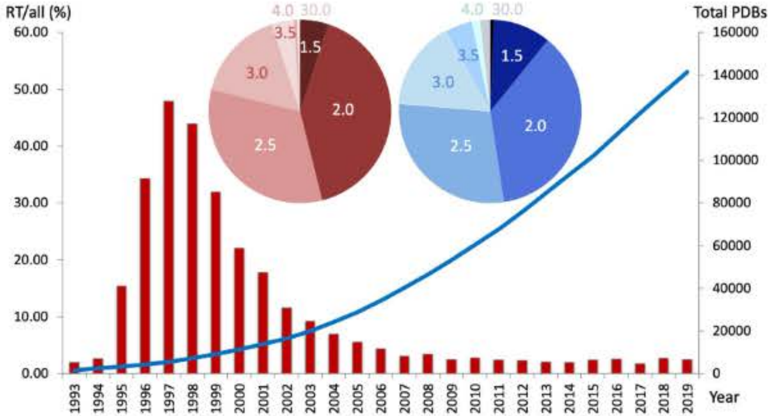




- The advent of CryoCrystallography has boosted the determination of novel structures over the last decades
- It facilitated the advent of robotics
- Remote operation
- Automation
- Paved the way for radiation damage studies, better data collection strategies
- But…

BEAMLINE WISHLIST

- High Flux density
- Timed beam
- Synchronise exposure time with other events
- Adapt different sample environment
- Record accurate intensities
- Facilitate data handling
- Facilitate sample preparation on site

EBSL8 IN A NUTSHELL

- Room temperature Serial crystallography experiment
- Extremely high flux with exposure time in µs range and high repetition rate
- Adapt different sample environments and crystal delivery systems
- A dedicated sample preparation laboratory and data analysis area
- Study structural evolution of irreversible enzymatic reaction triggered by different methods
- Study and characterisation of radiation damage at very high flux

Photobiology

Study light activatable biological processes

Investigate light dependent biochemical reaction

ID29

Serial and time resolved crystallography

Enzymology

Study enzymatic reaction in crystals

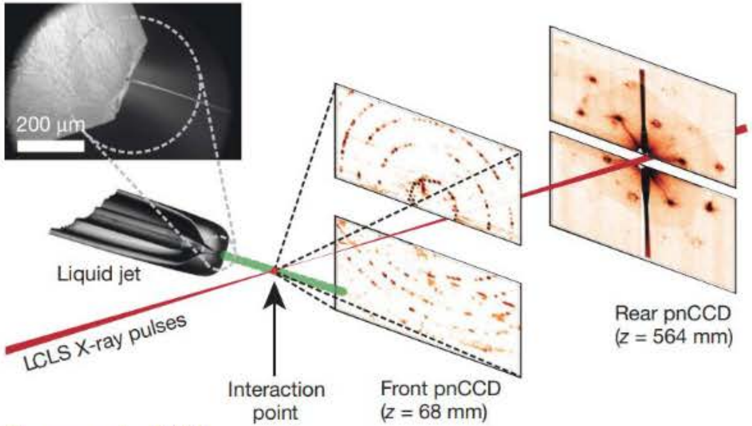
Enzyme design and repurposing by synthetic biology

Drug design

Exploit room temperature fragment screening

Identify time dependant structure:ligand complexes

SERIAL FEMTOSECOND CRYSTALLOGRAPHY

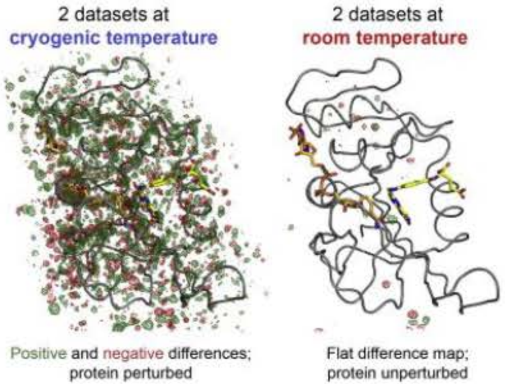


Chapman et al 2011

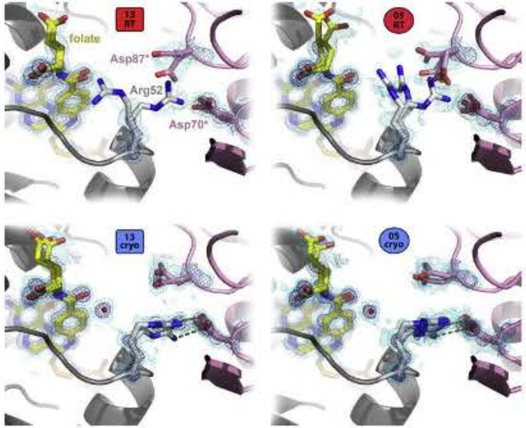
Boutet et al 2012

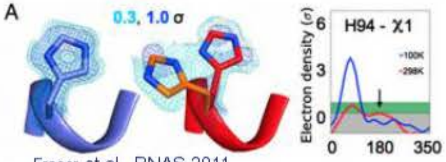
- Diffraction before destruction
    - Radiation damage "free"
- Possible to study nano-to-micro crystals
- Determine structure at Room temperature
- No cryo-protectant artifacts
- New methods and software to index and integrate data
- Diffraction is from "still" crystals (no rocking)
- Thousands of indexed pattern are necessary to reconstruct
- New data quality indicators

ROOM TEMPERATURE DATA COLLECTION

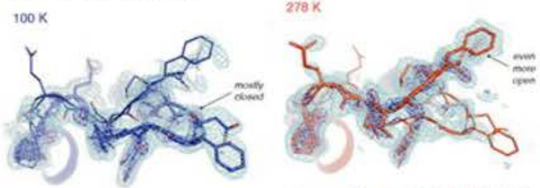


Keedy et al 2014



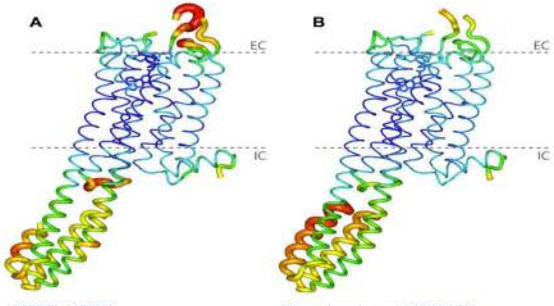


Fraser et al., PNAS 2011



James Fraser, ESRF UM2016

- CryoMX was one of the keys for MX popularity
- 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
- Because of radiation damage, serial crystallography is the most valuable route to obtaining RT structures.



Liu et al. 2013

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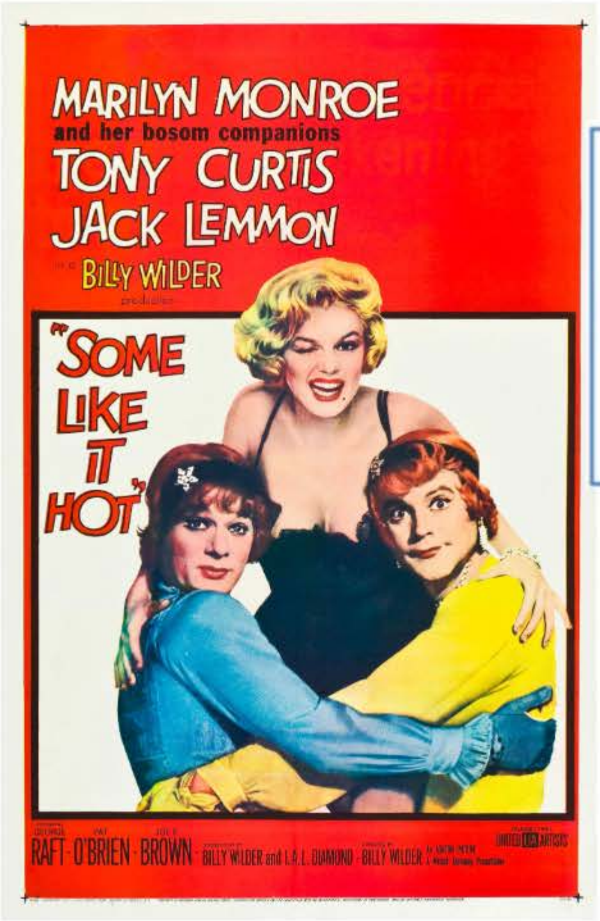
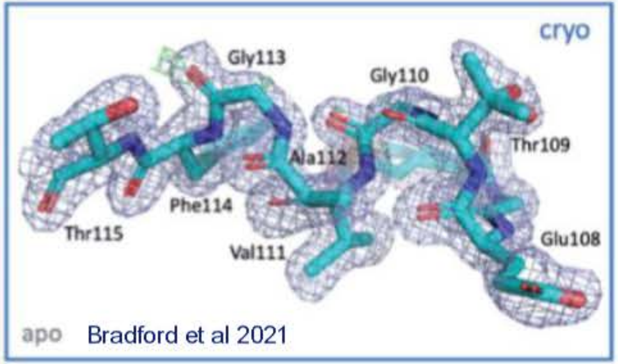
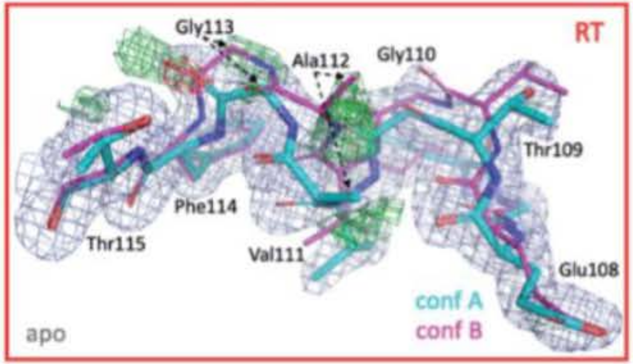
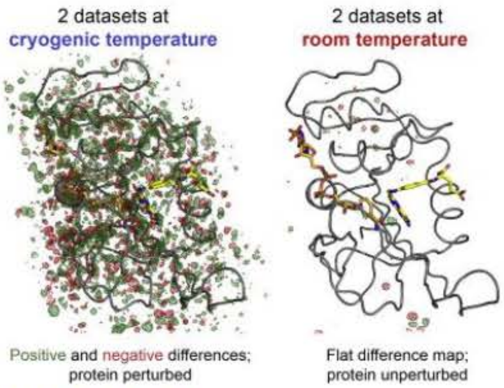
- They might hide **functional conformations** and prevent binding of substrates or inhibitors



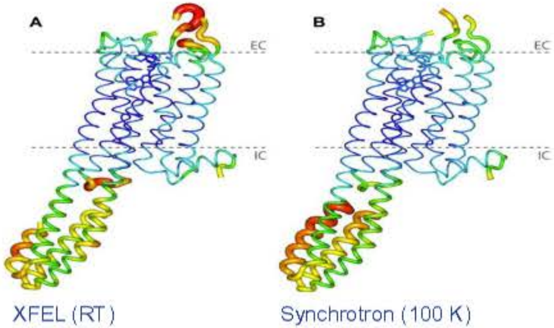


Bradford et al 2021

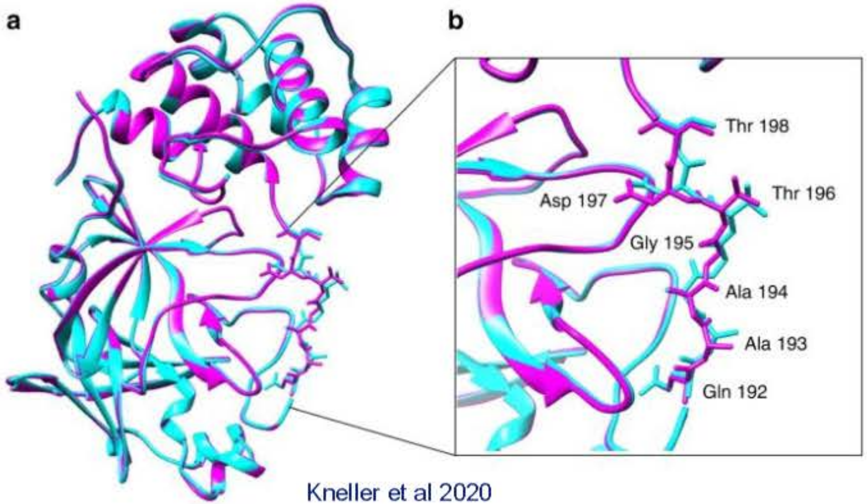




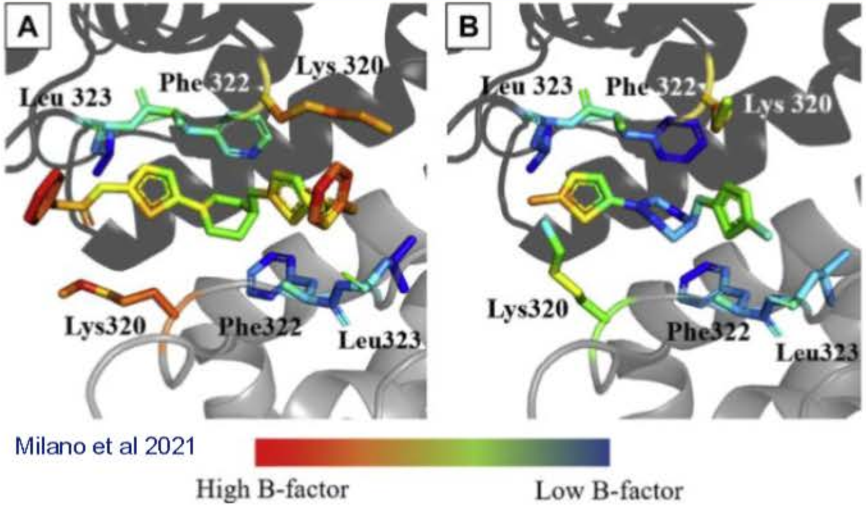
Keedy et al 2014



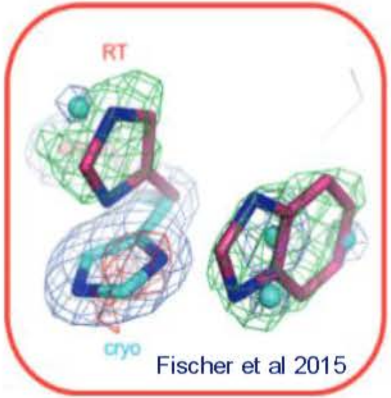
Liu et al. 2013



Kneller et al 2020



Milano et al 2021



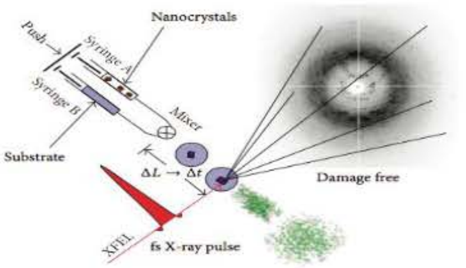
Fischer et al 2015

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

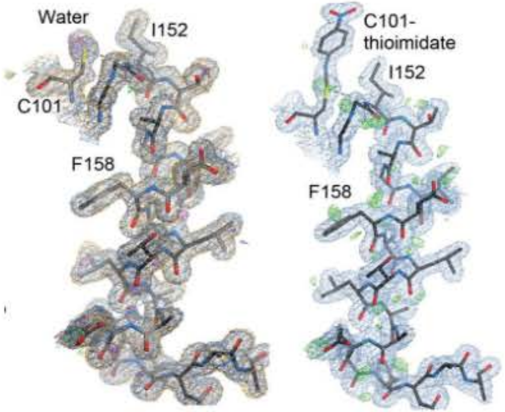
SERIAL CRYSTALLOGRAPHY

Hydrated microcrystals at room temperature can:

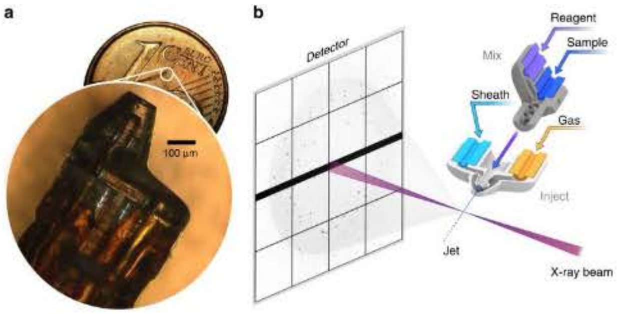
- be activated
- be soaked



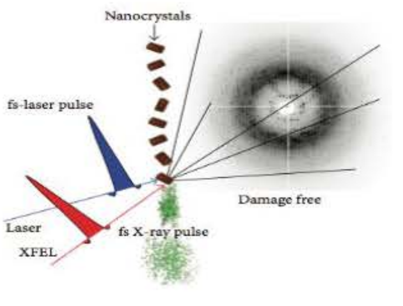
Schmidt, M. 2013

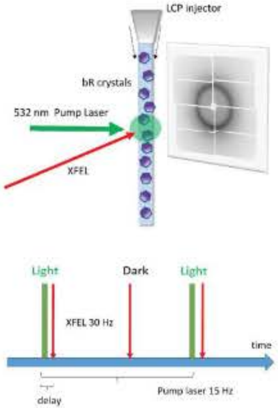


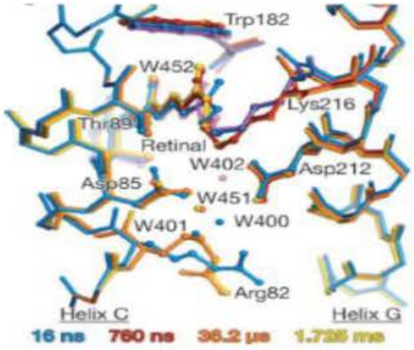
Dasgupta, et al. 2019.



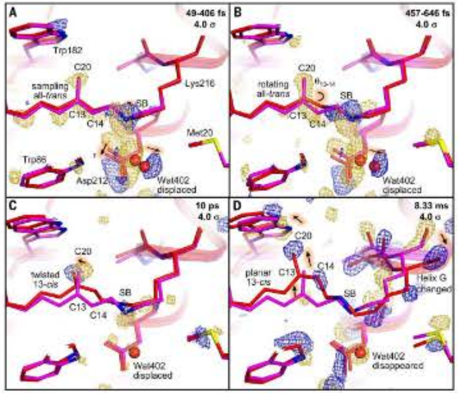
Knoška et al 2020







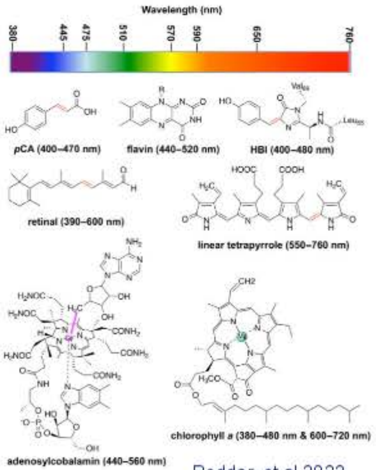
Nango et al. 2016



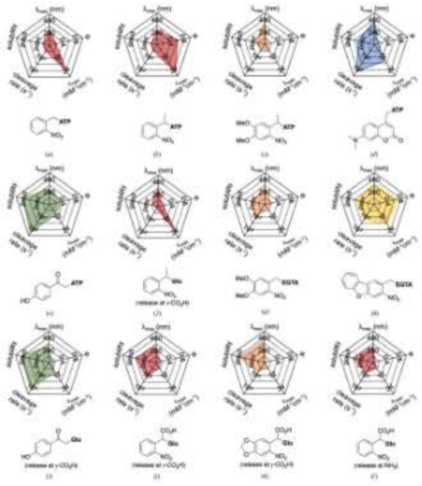
Nogly, et al. 2018

PROTEIN DYNAMICS IN CRYSTALS

- Pump & probe
  - Photoactivatable **proteins** (UV-vis)
  - Photoactivatable **ligands** (UV-vis)
    - Cleavable **cage** compounds
    - Activatable cofactors
  - **Temperature** jumps (IR)
    - Protein-protein **complex** dissociation
    - Protein-ligand dissociation



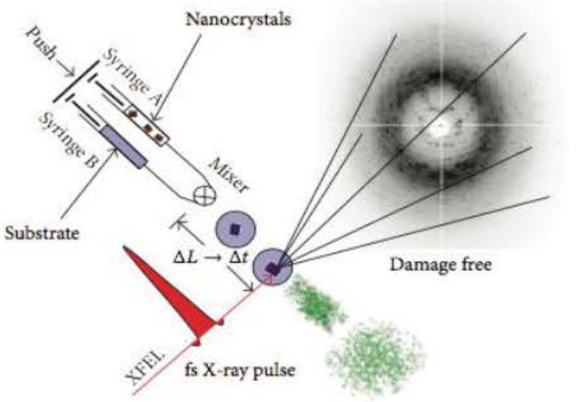
Poddar, et al 2022



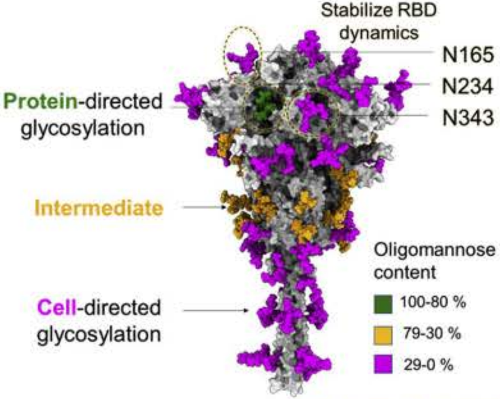
Monteiro, et al 2021

PROTEIN DYNAMICS IN CRYSTALS

- Ligand and buffer mixing
  - **Drug** binding
  - **Enzymatic reaction** in crystals
  - **pH changes**
  - Development of new more **efficient sample delivery for mixing** is crucial

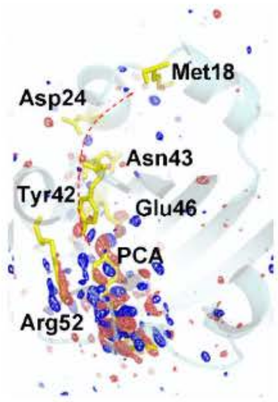


- But there is already demand for experiments
  - At (real) **physiological** temperature
  - In **anaerobic** environment for oxygen sensitive samples
  - **Glycoproteins** are a greatly (ri)-emerging field
  - More will come…

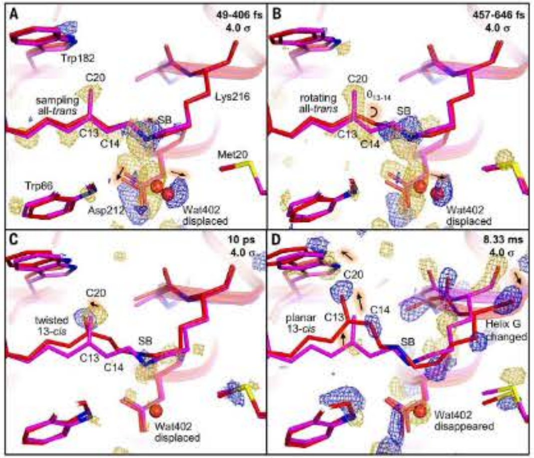


TIME RESOLVED STUDIES

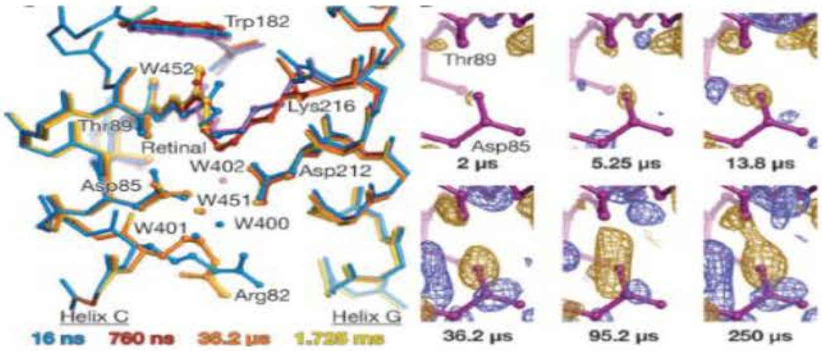
- New pathways for time resolved studies
- At 3rd generation synchrotron were mainly limited to Laue experiments
- Now serial crystallography permits to overcome damage issue and collect multiple time points



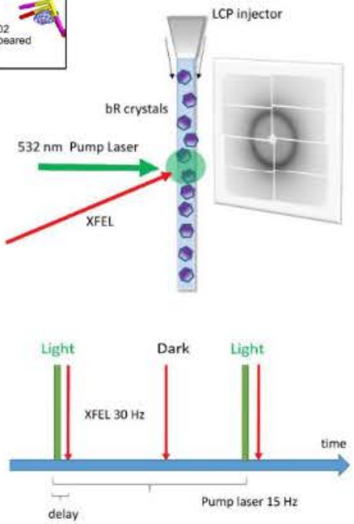
Tenboer et al. 2014.

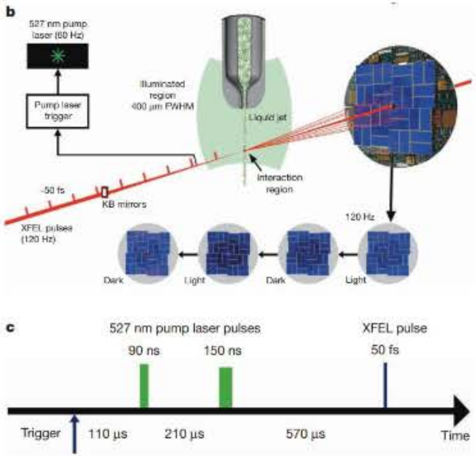


Nogly, et al. 2018



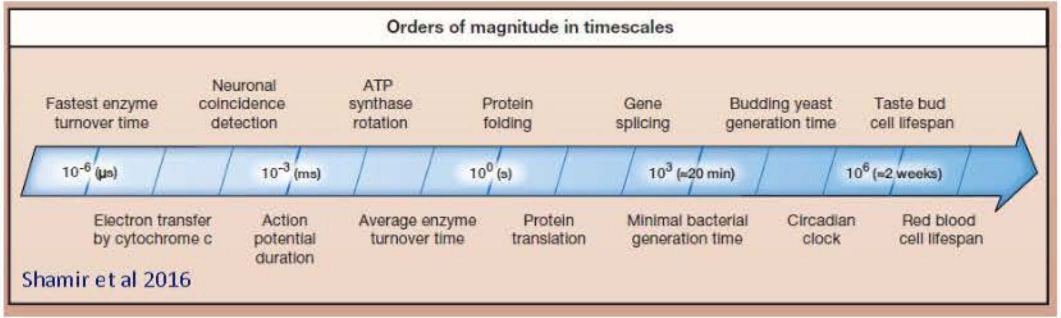
Nango et al. 2016

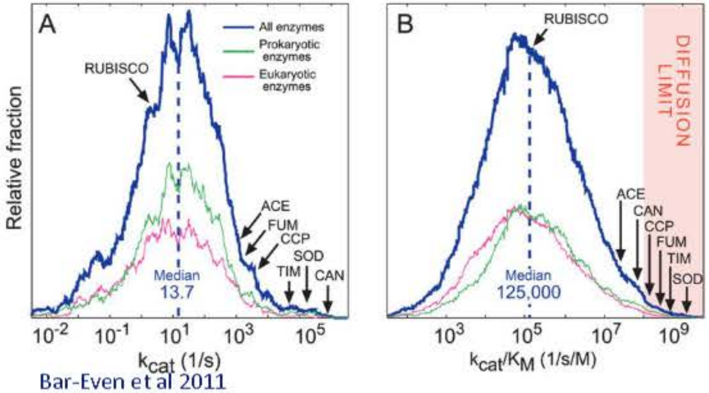


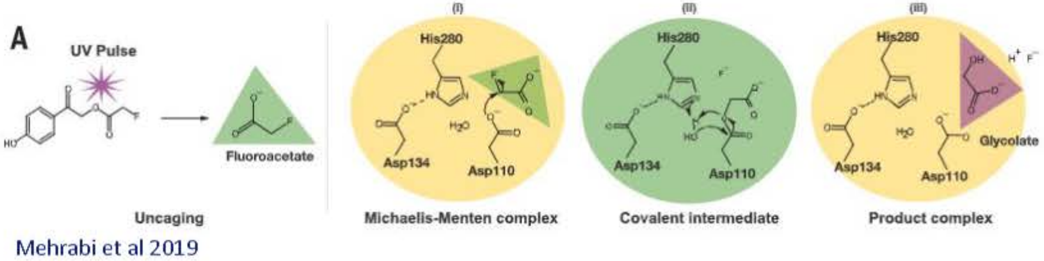


Kupitz et al 2014

TIMESCALES IN BIOLOGY





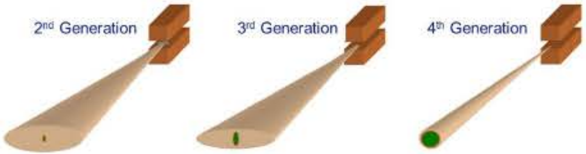


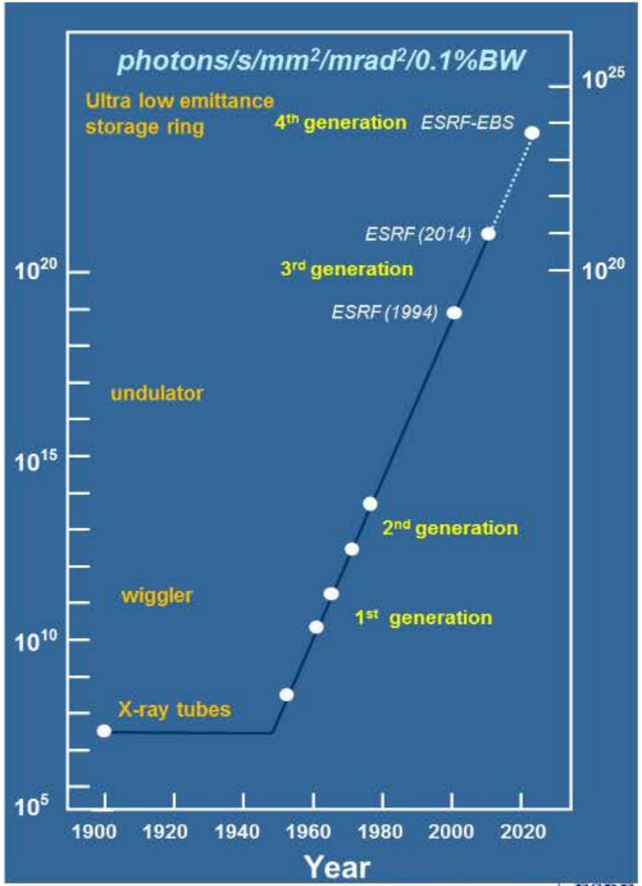
- Optimising the acquisition time in the micro-to-millisecond time range allows to study a waste majority of enzymatic processes
- The development and the use of photoactivatable cage compounds expands the processes that can be studied and define accurately t=0

- 3rd generation synchrotrons are mostly limited to milliseconds due to detector and available flux at the sample position
- Serial crystallography permits to overcome damage issue and collect multiple time points using a pulsed source
- 4th generation allow for a x10 flux and x10^5-10^6 flux density
- ID29 optical layout was designed to tackle this time resolution

ID29 WITH EBS

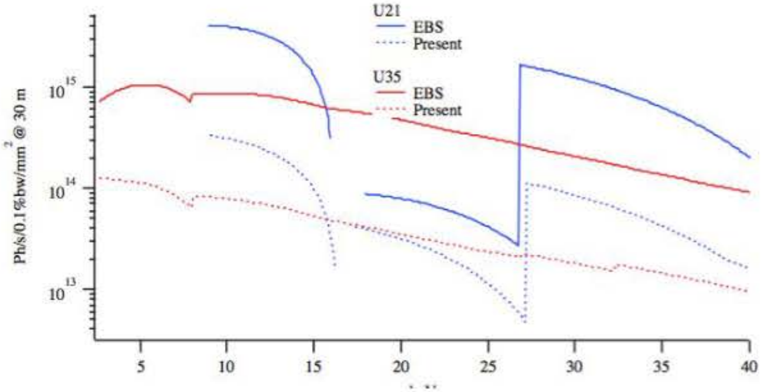
Parameter	ESRF low Beta (ID29)	ESRF EBS
Electron beam energy [GeV]	6.04	6
Nominal current [mA]	200	200
Relative rms energy spread of electron beam []	0.001	0.00095
Horizontal emittance [nm]	4	0.132
Vertical emittance [pm]	5	5
Horizontal beta [m]	0.35	6.9
Vertical beta [m]	2.95	2.65
Horizontal Dispersion [m]	0.0308	0.00175
Horizontal rms electron beam size [μm]	48.5	30.2
Horizontal rms electron beam divergence [μrad]	106.9	4.37
Vertical rms electron beam size [μm]	3.84	3.6
Vertical rms electron beam divergence [μrad]	1.3	1.38



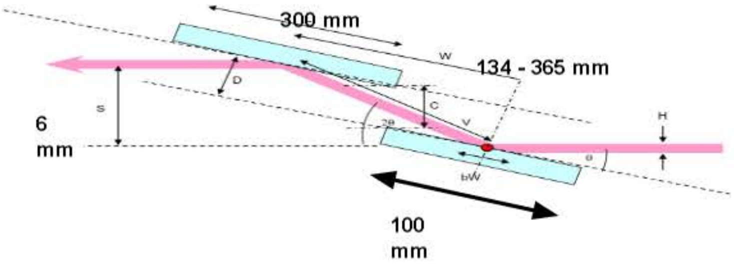


SOURCE - FLUX

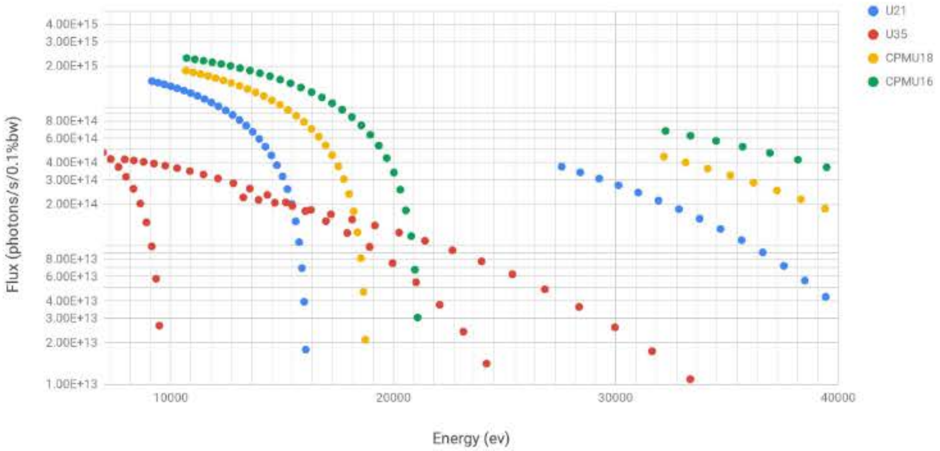
- The lower horizontal emittance increases by > 10x the useful flux with the same source configuration (old ESRF vs. ESRF-EBS)



- Initial operation with current undulator (IV U21)
- The upgrade of the source to CPMU16 will increase the energy range and the flux (particularly at high energy)
- 10-20 keV continuously tunable + 35 keV

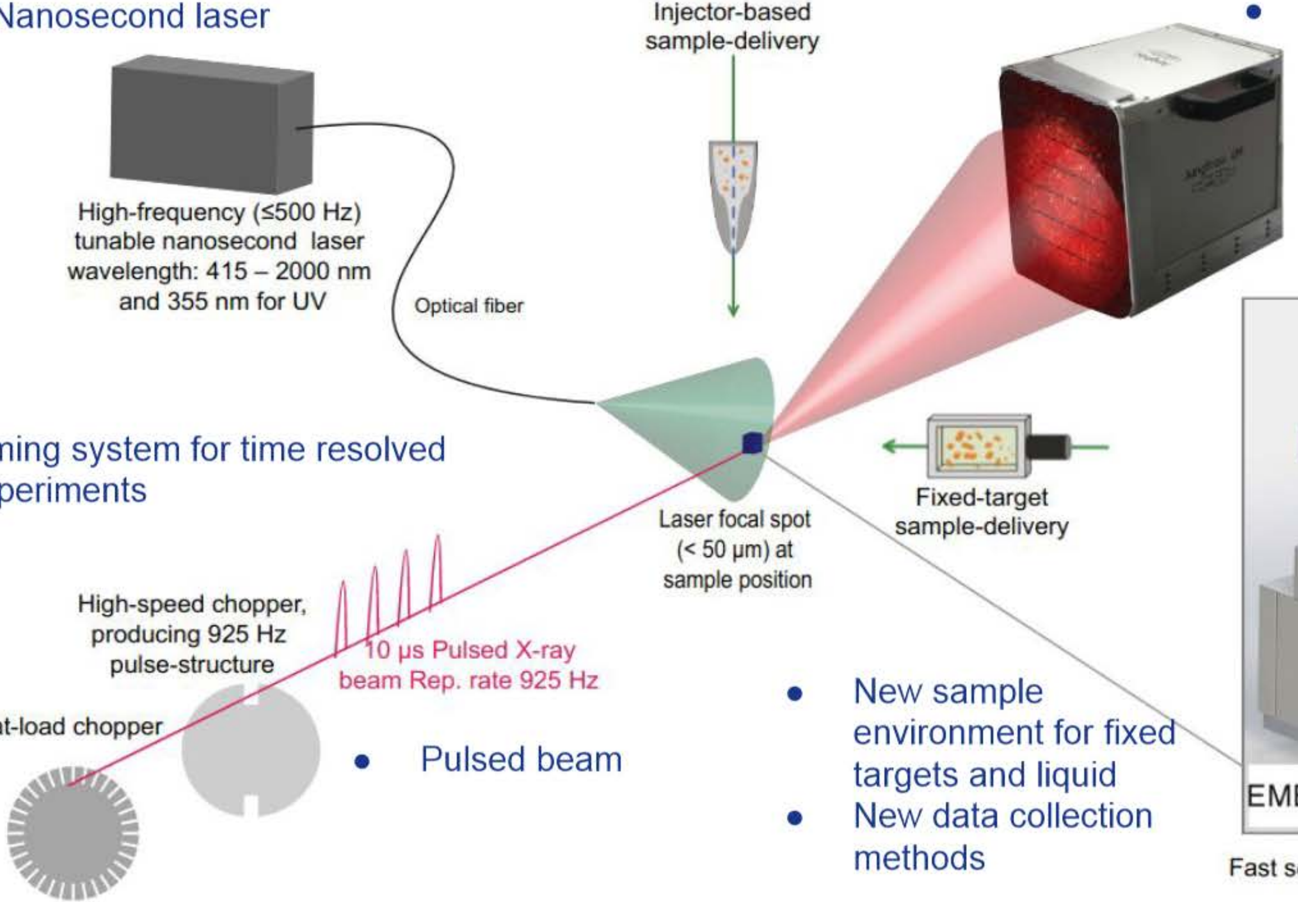


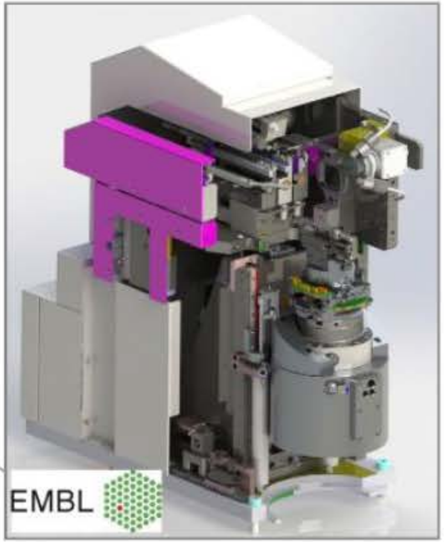
Flux at 30 m 1x1 mm slits



A NEW EXPERIMENTAL SETUP

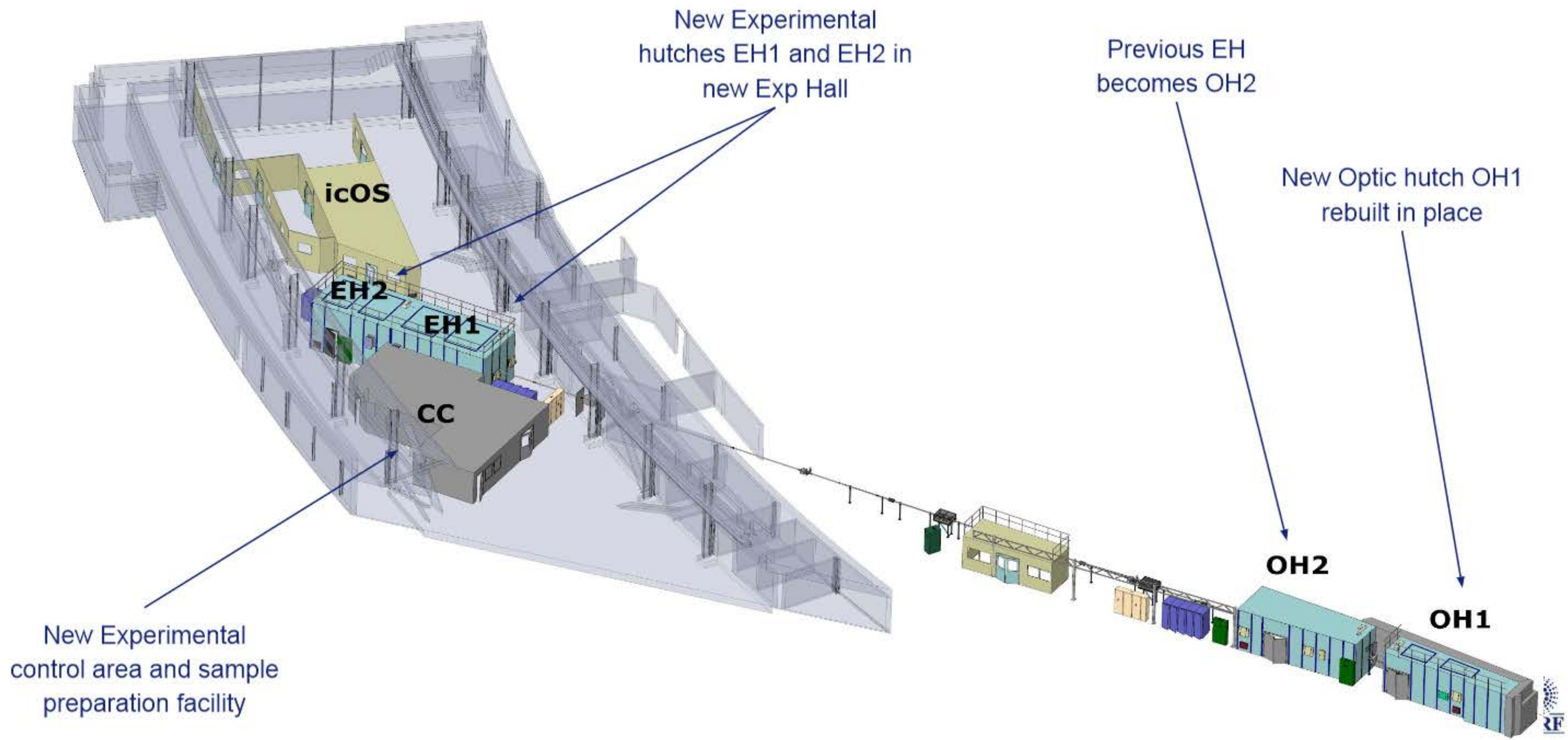
- Nanosecond laser
- New integrating detector
- Timing system for time resolved experiments
- Pulsed beam
- New sample environment for fixed targets and liquid
- New data collection methods

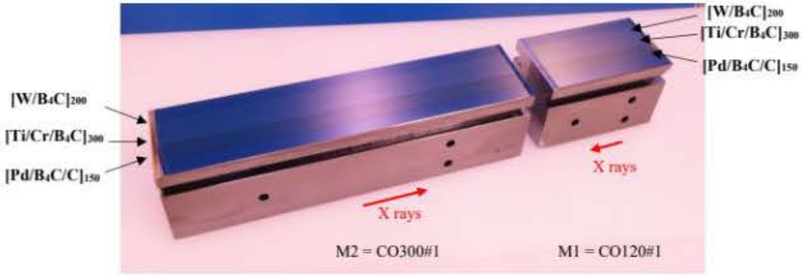


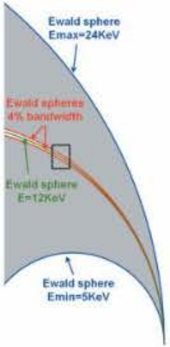


Fast scan enabled by SSX-head (1 KHz)

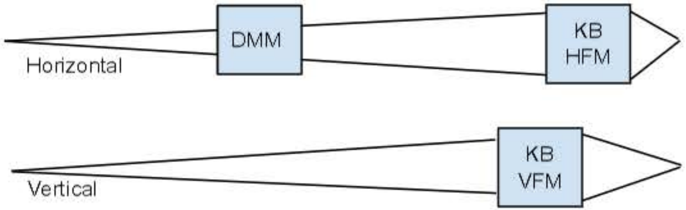
BEAMLINE LAYOUT





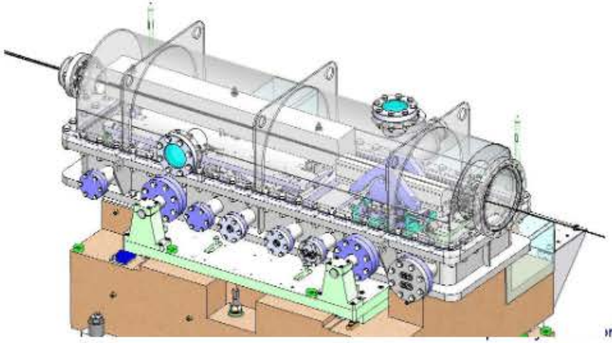


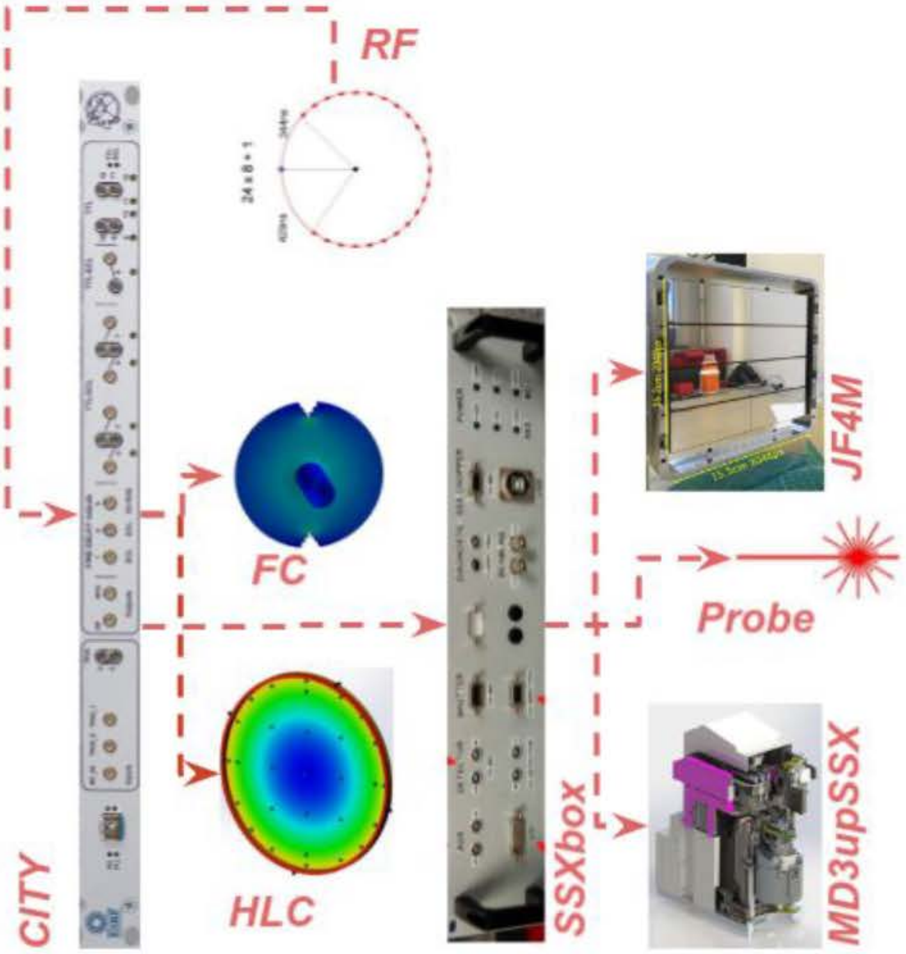
- Sample at 107 m from source
- Working distance to sample 500 mm
- Beam divergence 0.7 x 1.9 mrad (VxH)
- Smallest spot size 0.5 x 0.6 µm (VxH)
- Beam resizing by tuning incident angle



- A multilayer monochromator system was designed to increase bandwidth (higher flux and ticker Ewald sphere)
- Adjust bandwidth by changing stripe
- The multilayer monochromator permits to deliver higher flux in larger bandwidth
- Three stripes are present to cover whole energy range 10-20 keV (+35 keV) with 0.3% and 1% bw

- **Elliptical KB mirrors**
    - HFM slope error < 0.1 µrad
    - VFM slope error < 0.05 µrad

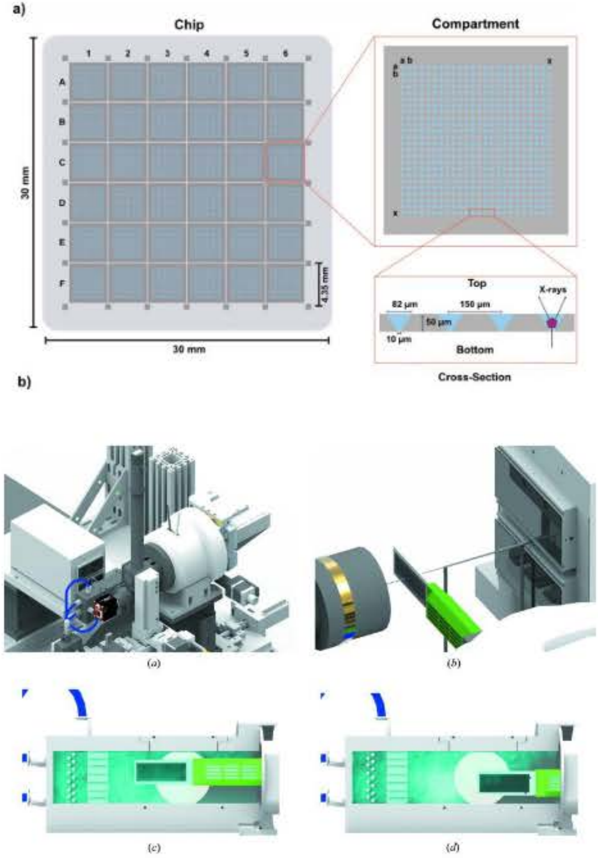




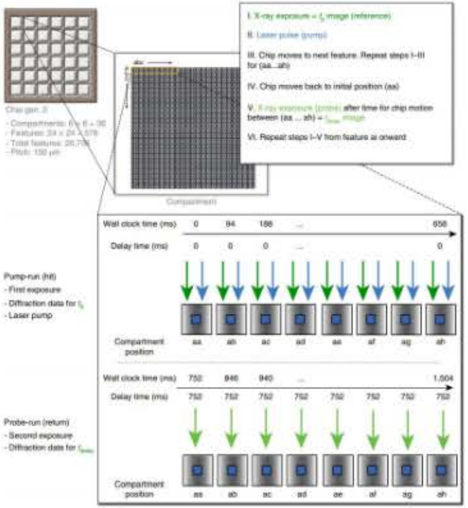
- A new developed timing system synchronises every step of the acquisition with the radiofrequency of the storage ring
- CITY and SSXbox are the two hearts of the system
- Heatload and Fast chopper are synchronised
- SSXbox propagate the clock to the data acquisition devices, MD3upSSX, X-ray detector and additional triggerings
- **X-ray pulses** of tunable length from 10 to 90 microseconds
- Synchronous triggering of external probes

FIXED TARGETS

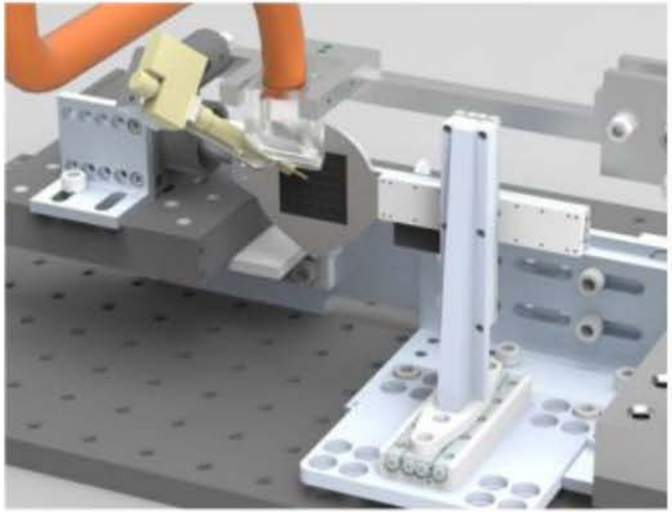
- Alternative to injectors are represented by fixed targets, where microcrystals are sitting in a regular arrays
- They can be used for light triggered time resolved experiments
- Collect multiple time points with the Hit&Return method
- Or in combination with nano-pipetting systems, to add ligands, substrates, ...



Tolstikova et al 2019

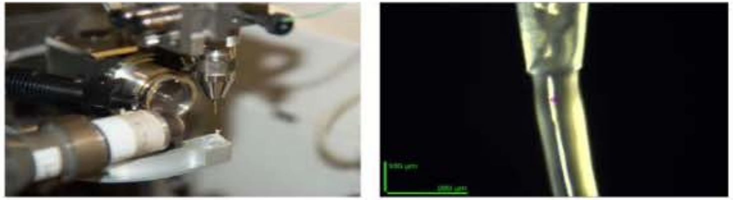


Schultz et al 2018

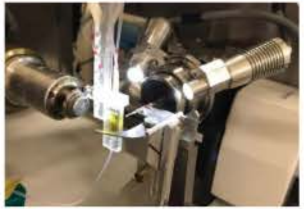


Mehrabi et al 2019

SAMPLE DELIVERY - LIQUIDS

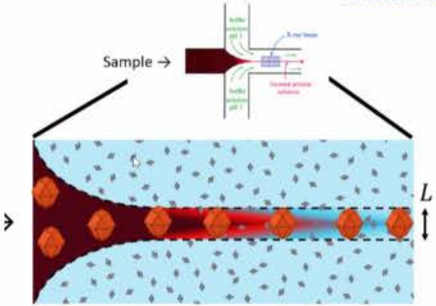


von Stetten et al, 2020



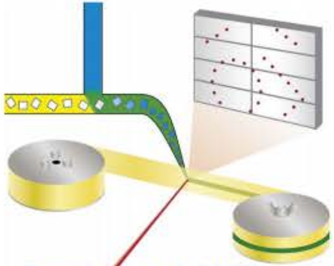
Monteiro et al 2020

Botha et al, 2015



Katz, et al.

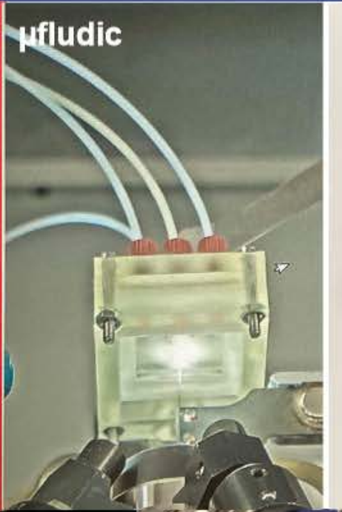
Roessler, et al 2013

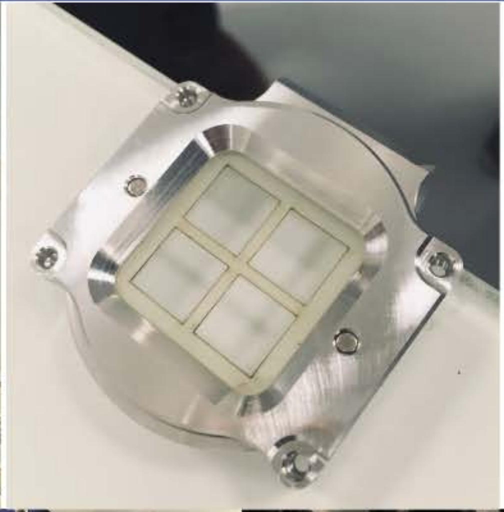


Beyerlein, et al. 2017.

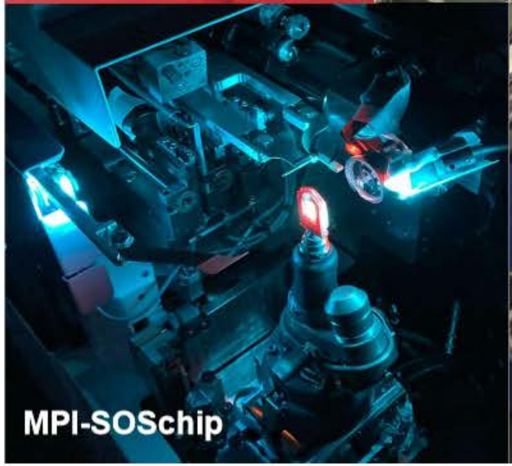
- In liquid sample delivery **crystals travels** in front of the beam
- Diffraction is recorded when **X-ray pulse intercepts a crystal**
- They include:
  - High viscosity media
  - Microfluidic chips
  - Capillaries
  - GDVN
  - Convey belts
- The choice depends on the crystals and on the goal of the experiment

MORE SAMPLE DELIVERY METHODS

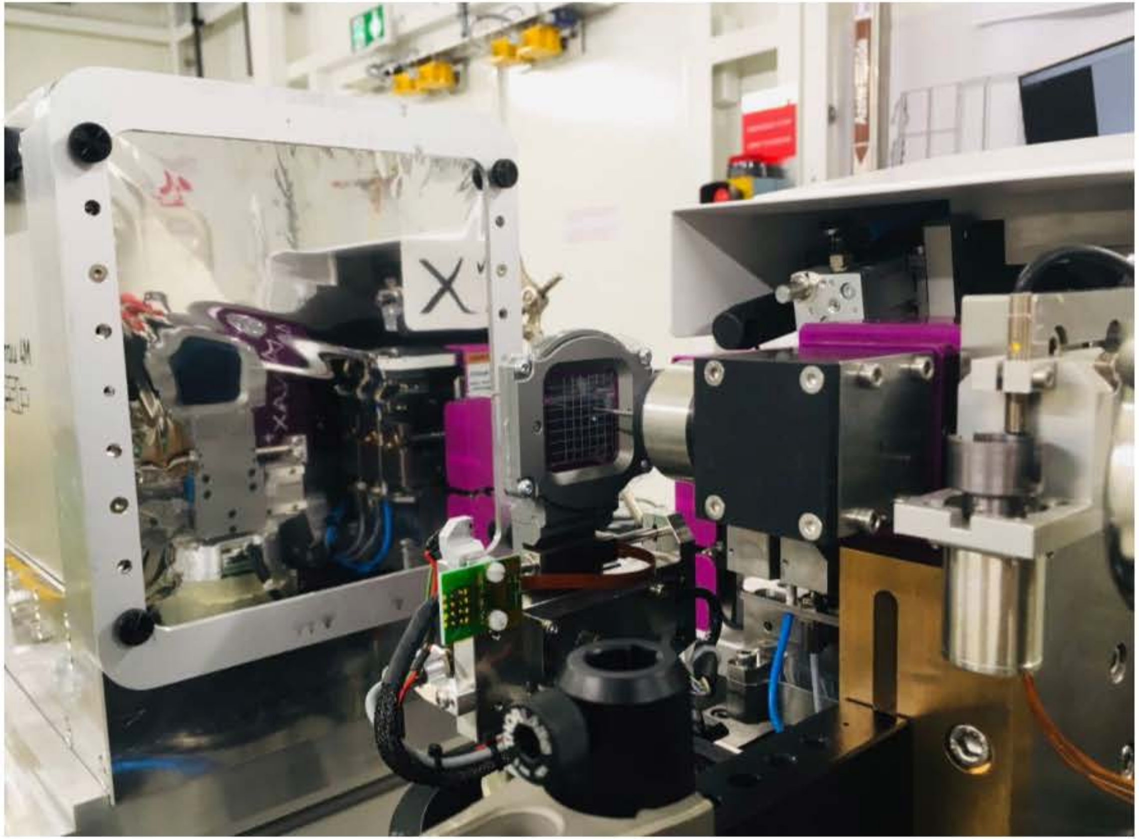


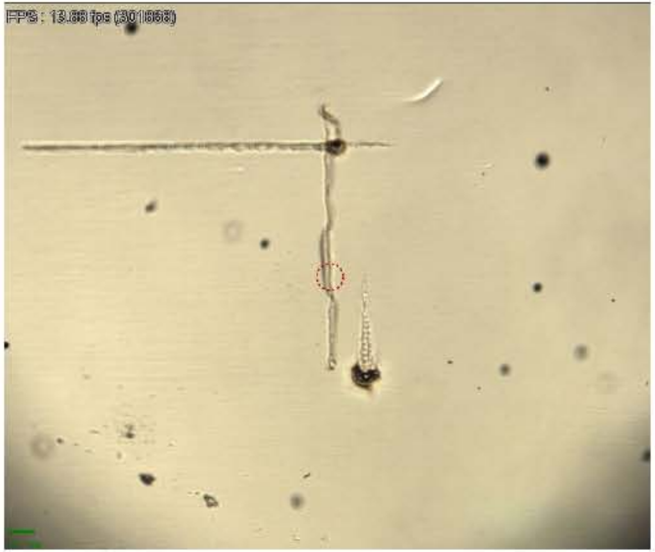


- Sample delivery methods is the heart of future SSX experiments
- We are developing microfludics, fixed targets
- We can operate three different HVE injectors



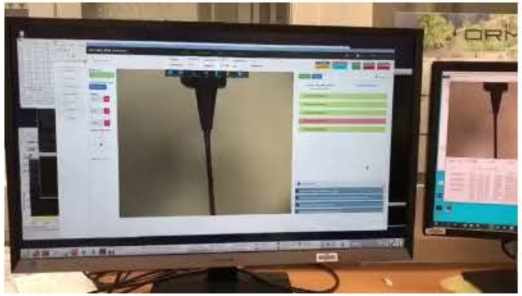

ARINAX
Jungfrau
MD3-UP
EMBL



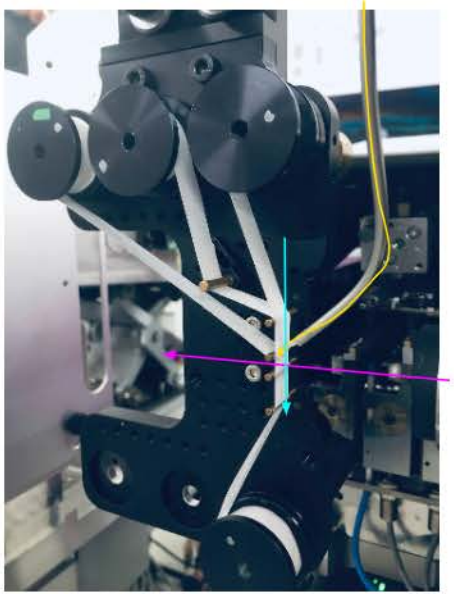


- KB with fixed curvature
- Measured beamsize ~ 2 x 2 um
- KB protected by heat load chopper
- Monochromator and beam position very very stable

LAST RUN - TAPE - HVE - FIXED TARGET - ALD

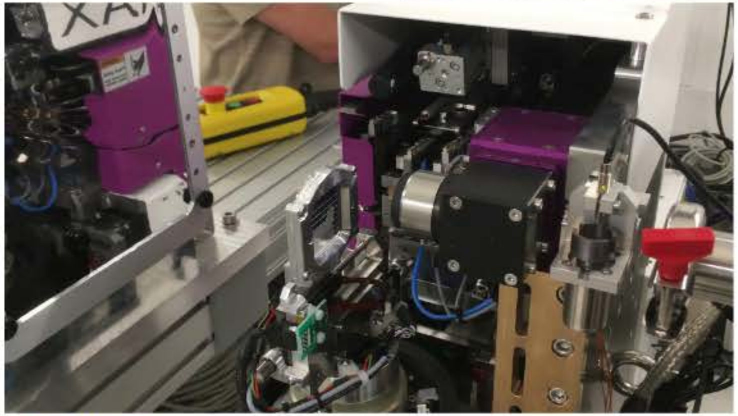


ALD - PSI development



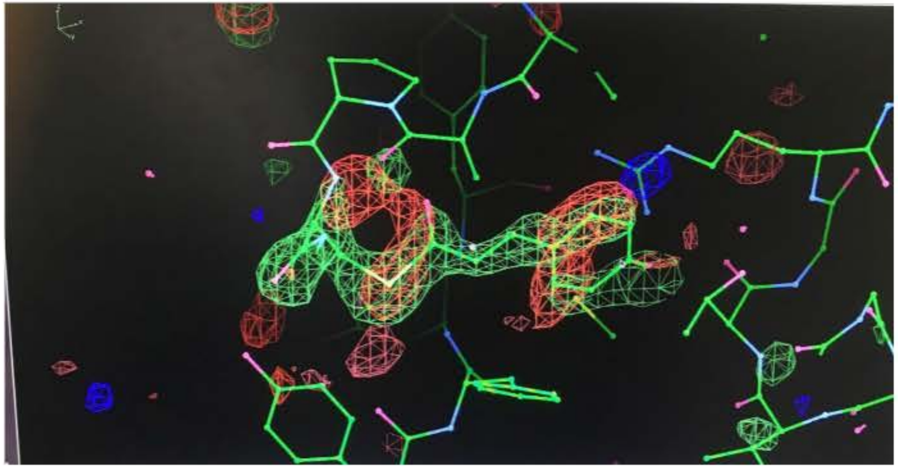
Tape4SSX BMBF collaboration

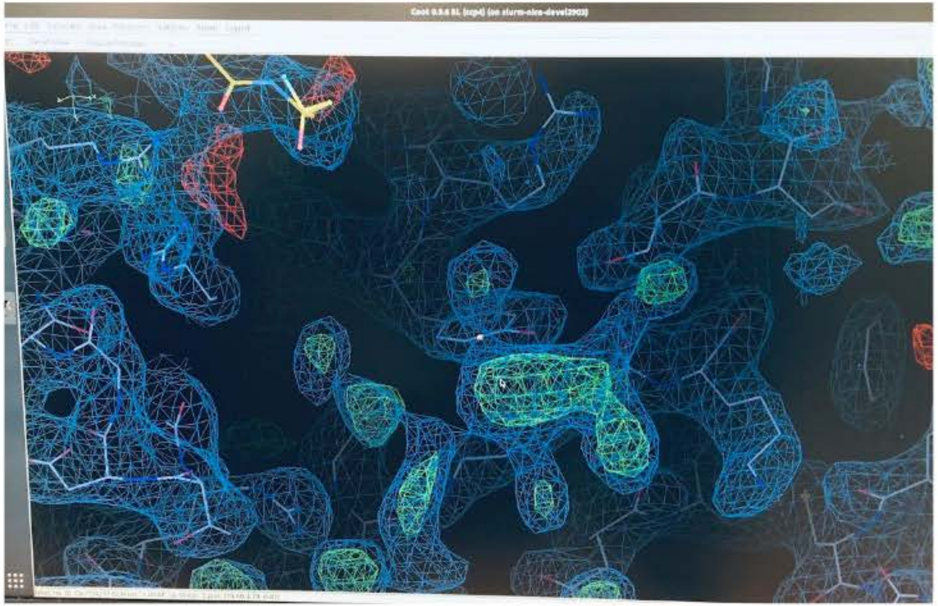
HVE



Fixed target

INITIAL RESULTS

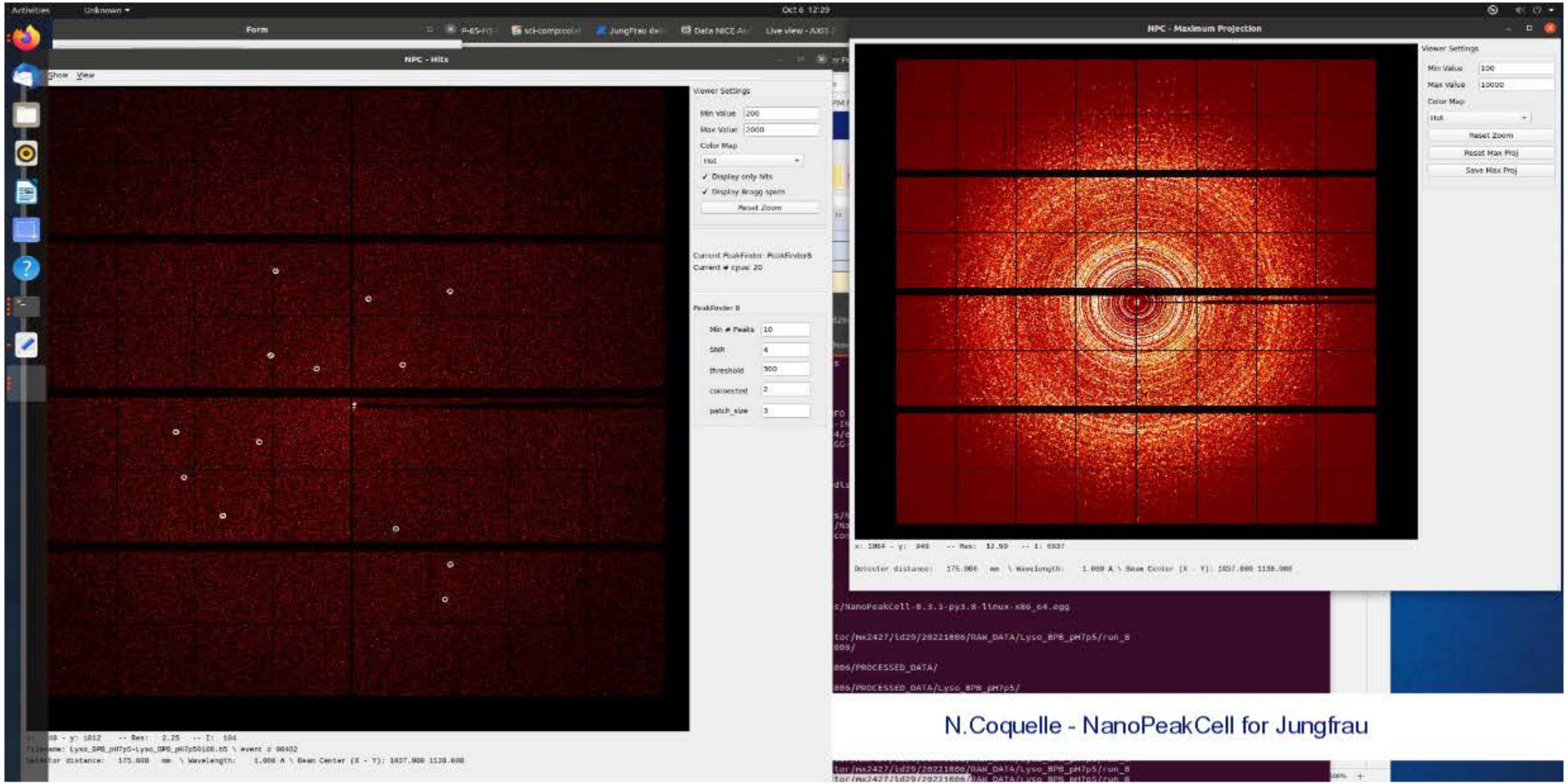




- Photoswitchable molecule

- Protein - ligand interaction

DATA ANALYSIS



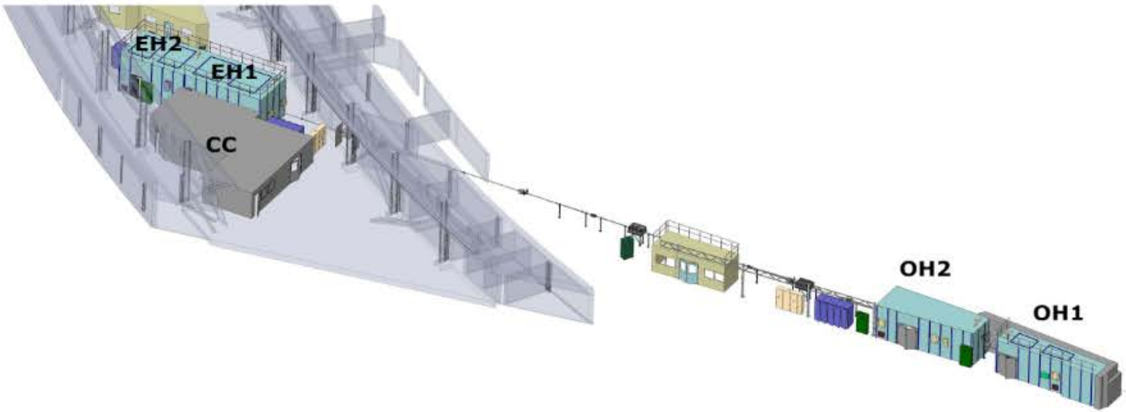
N.Coquelle - NanoPeakCell for Jungfrau

- New needs for Data processing
  - Hit finding (NanoPeakCell) → indexing (CrystFEL) → integration (CrystFEL)

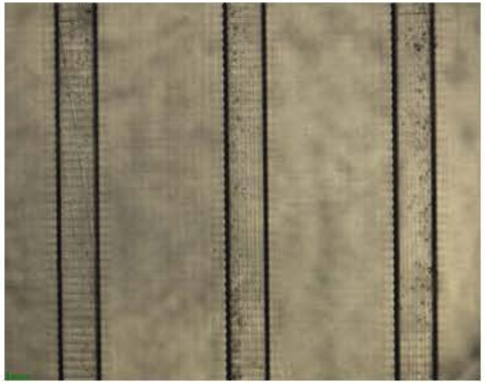
ID29 IN A NUTSHELL

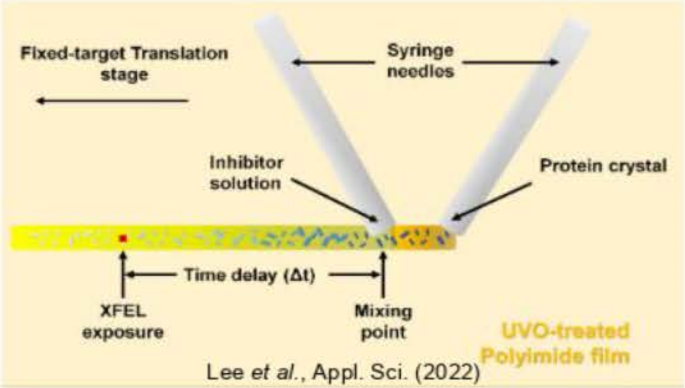
- The **first world beamline** dedicate to Time-resolved Serial crystallography at room temperature at 4th generation synchrotron
- A **reference** and inspiration for similar beamlines planned or under construction at other synchrotrons
- A new design with **unmatched beam** characteristics
- Equipped with **latest generation synchronisation** devices
- A **new diffractometer** for fixed target and sample injectors experiments
- Fully tunable **nanosecond laser** at high repetition rate
- Latest generation **integrating detector** with automatic gain switching
- The perfect facility for serial crystallography **experts**
- A **user-friendly** companion for new users
- A sample preparation laboratory for initial testing and user training already accessible
- Porting automation to SSX

- **A new Structural Biology tool for**
  - time-resolved experiments
  - Serial crystallography
  - room temperature structures
  - Radiation damage studies

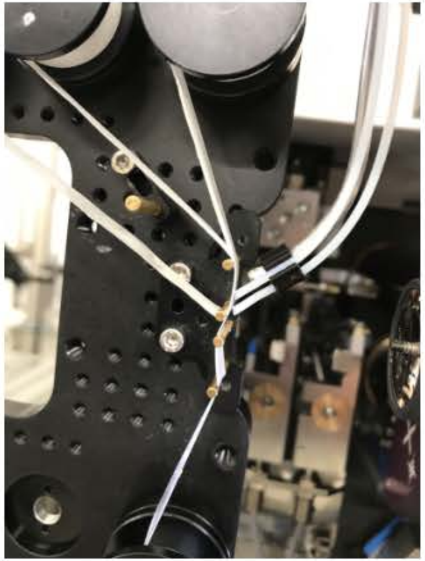


- In PubMed, the publications associated to TR-SSX experiments:
- ~70% are about photoactivation methods
- ~30% are about mixing methods
- Only <0.5% of proteins are naturally photosensitive (Monteiro *et al.*, Acta D (2021))
- It is necessary to develop mixing methods in order to be able to study a larger number of enzymatic reaction but also protein substrate/inhibitor binding.

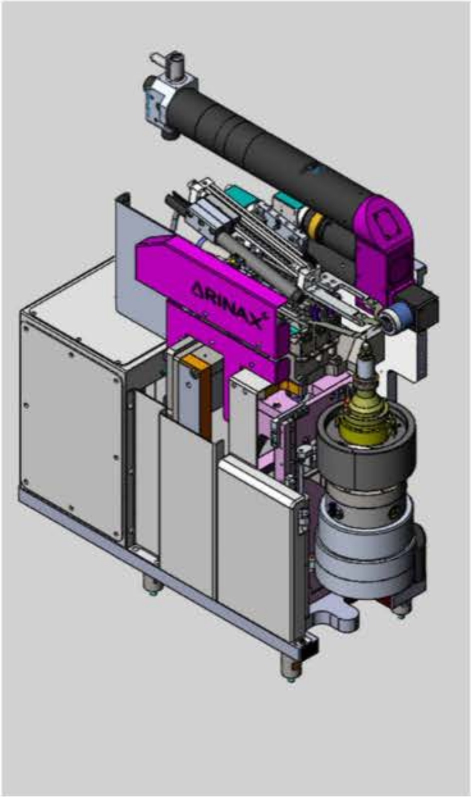


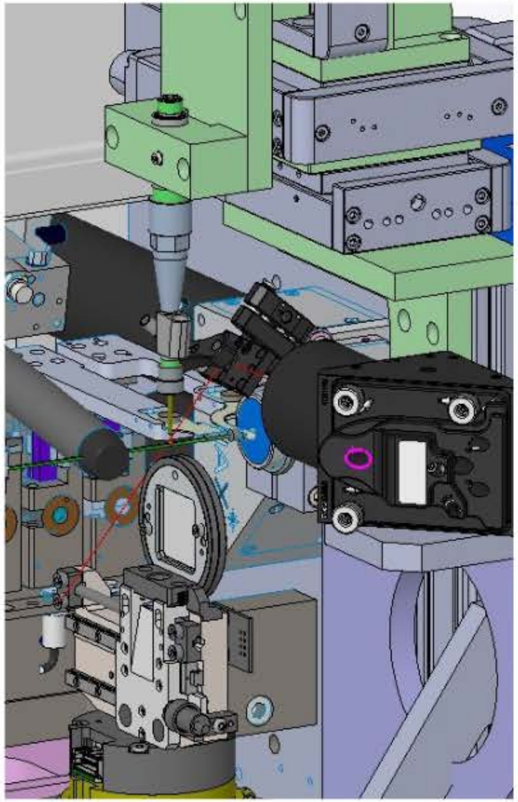


Lee *et al.*, Appl. Sci. (2022)



FUTURE DEVELOPMENTS - LIGHT ACTIVATION





- 400 - 2000 nm laser at 500 hz, 10 mJ max pulse energy
- New development injection through OAV
- And side focusing for injector operation
- Synchronised with experiment clock

Questions??

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