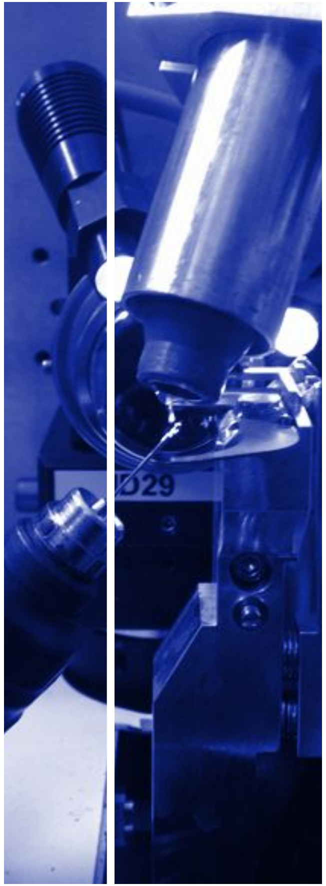
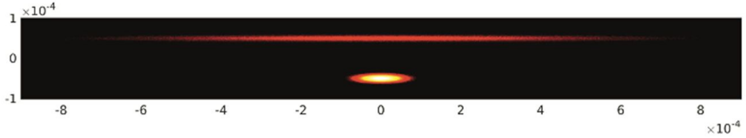
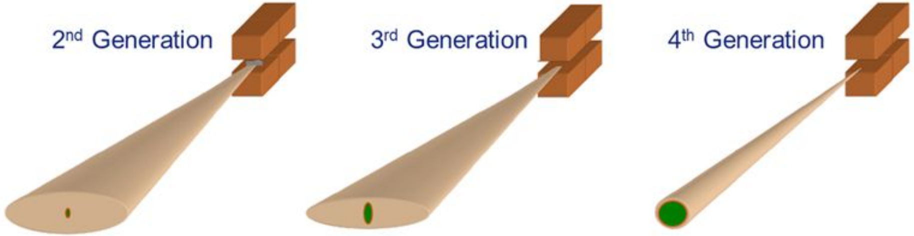


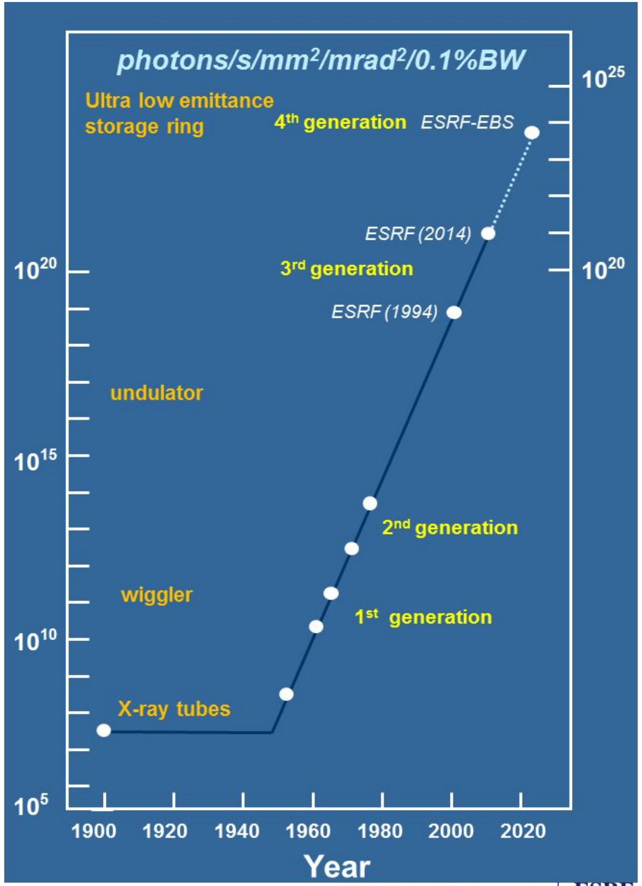
The New ID29: Planning and User access

Daniele de Sanctis - daniele.de_sanctis@esrf.fr
Shibom Basu - shbasu@embl.fr



- photon source **brilliance** (**x100**)
- **coherent** fraction of the photon beam (**x50**)

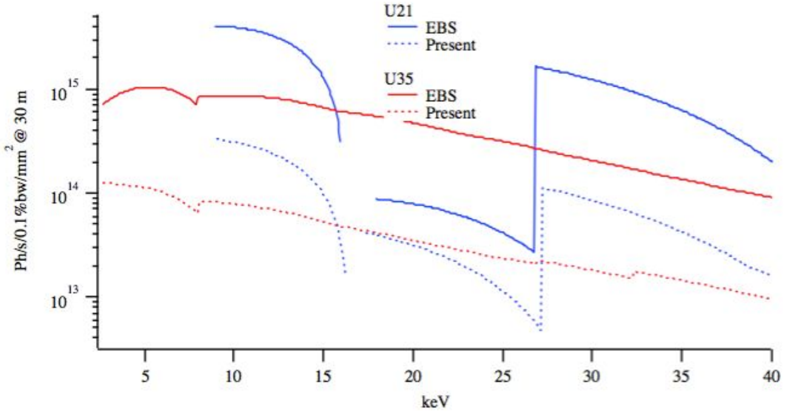




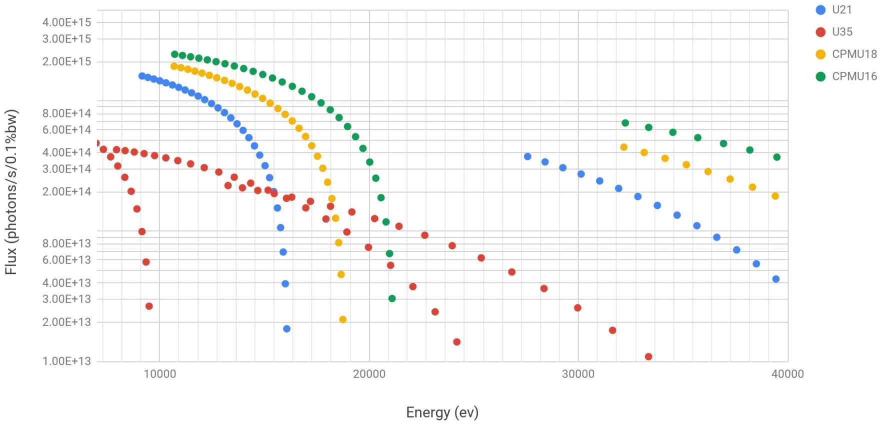
SOURCE - FLUX

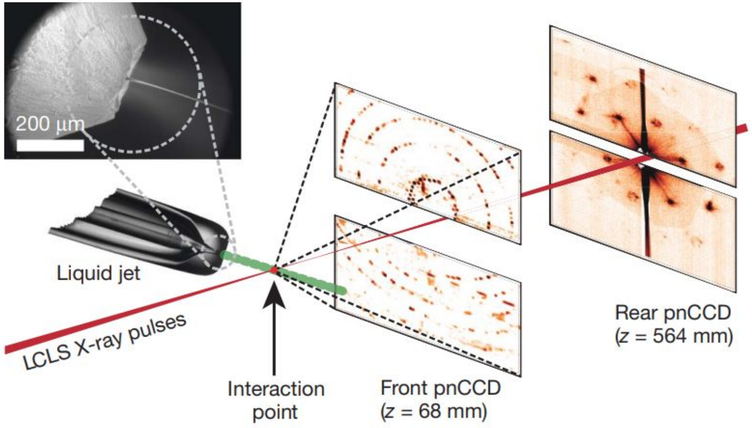
Parameter	ESRF low Beta (ID29)	ESRF EBS
Electron beam energy [GeV]	6.04	6
Nominal current [mA]	200	200
Relative rms energy spread of electron beam []	0.001	0.00095
Horizontal emittance [nm]	4	0.132
Vertical emittance [pm]	5	5
Horizontal beta [m]	0.35	6.9
Vertical beta [m]	2.95	2.65
Horizontal Dispersion [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

- The new storage ring brought a x30 reduction in H emittance
- Initial operation began with already available source
- Future configuration will have a permanently cryocooled undulator that will provide additional increase in flux and expand the energy range



Flux at 30 m 1x1 mm slits





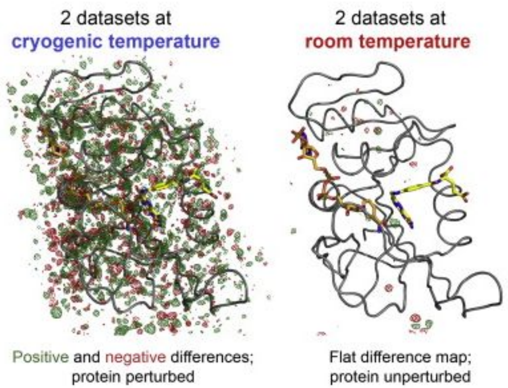
Chapman et al 2011

Boutet et al 2012

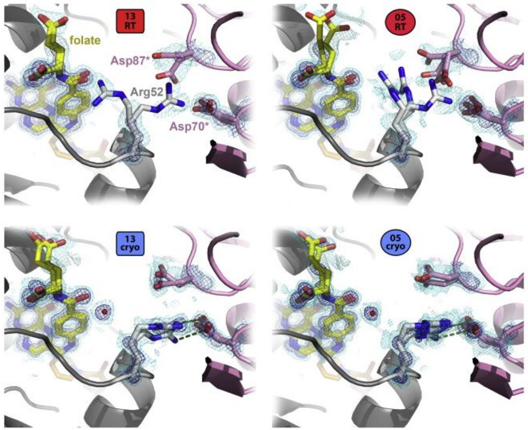
The advent of SFX opened new perspectives in macromolecular crystallography:

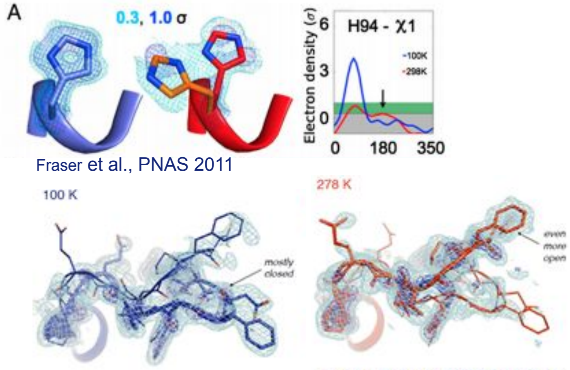
- Diffraction before destruction
- Outrun radiation damage at RT
- Possible to study nano-to-micro crystals
- Reveal structure without cryo-protectant artifacts
- Diffraction is from “still” crystals (no rocking)
- New methods and software to index and integrate data
- Diffraction patterns from thousands of crystals
- A new way to perform time resolved experiments

ROOM TEMPERATURE DATA COLLECTION



Keedy et al 2014

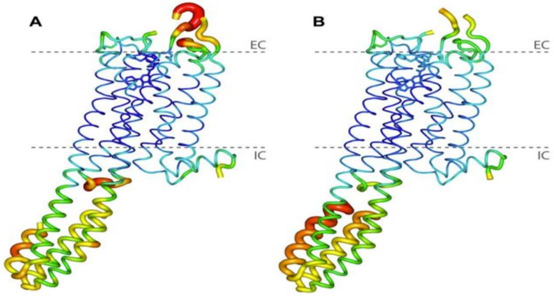




Fraser et al., PNAS 2011

James Fraser, ESRF UM2016

- 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.



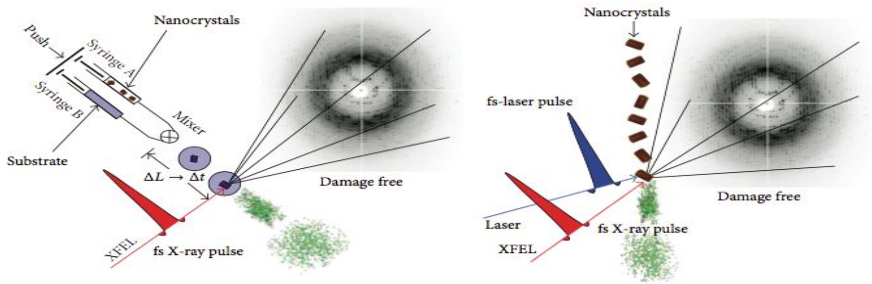
XFEL (RT) Synchrotron (100 K)

Liu et al. 2013

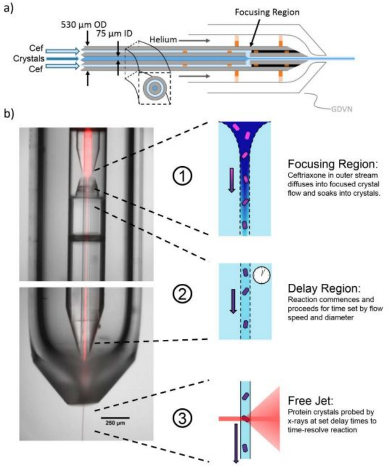
ROOM TEMPERATURE MICROCRYSTALS

Hydrated microcrystals at room temperature can:

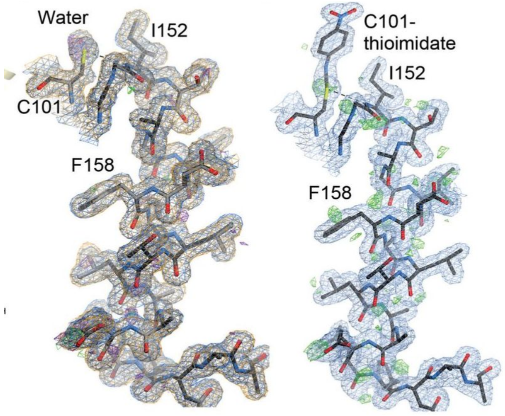
- be activated
- be soaked



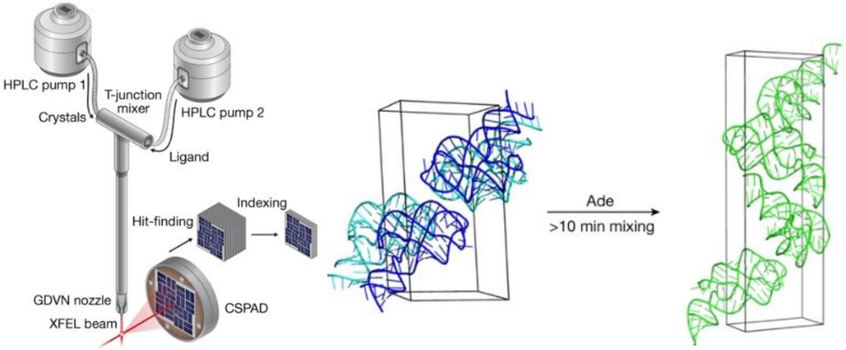
Schmidt, M. 2013



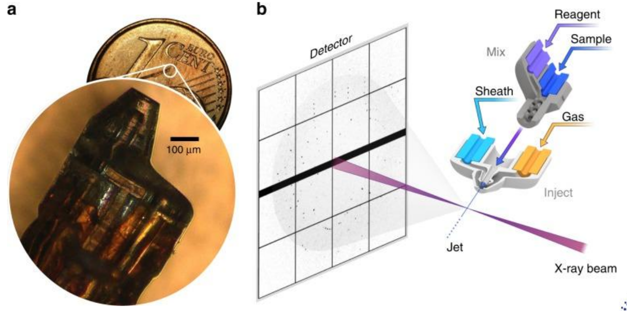
Olmos et al 2018



Dasgupta, et al. 2019.

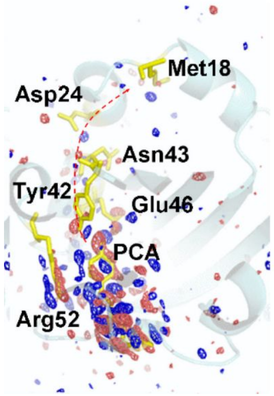


Stagno et al. 2017

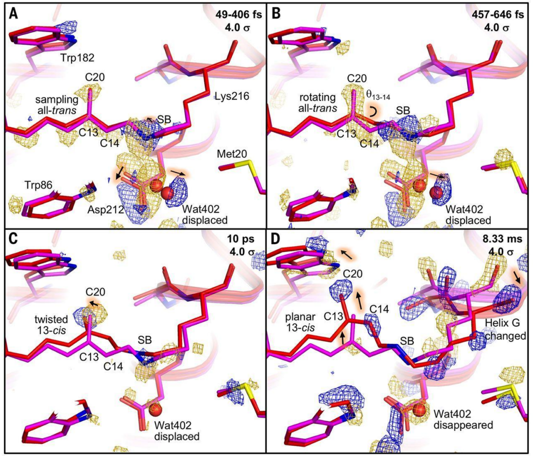


Knoška et al 2020

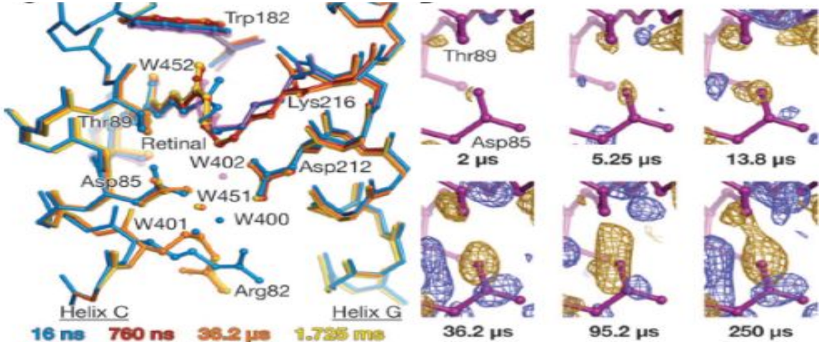
- New pathways for time resolved studies
- At 3rd generation synchrotron were mainly limited to Laue experiments
- Serial crystallography permits to overcome damage issue and collect multiple time points using a pulsed source



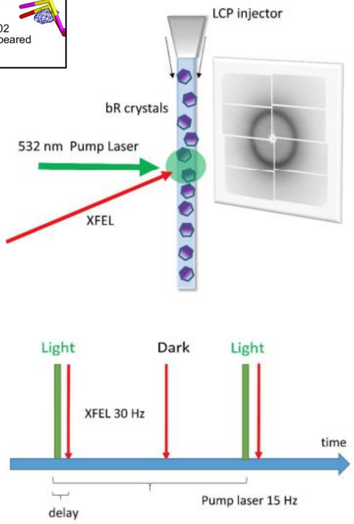
Tenboer et al. 2014.

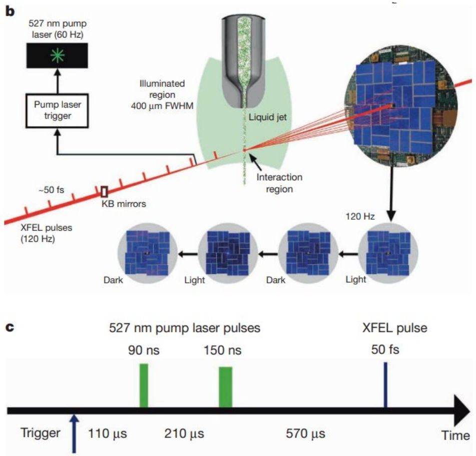


Nogly, et al. 2018



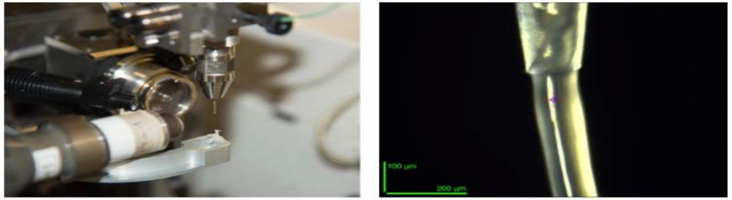
Nango et al. 2016



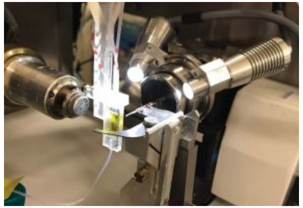


Kupitz et al 2014

SAMPLE DELIVERY AT SYNCHROTRONS

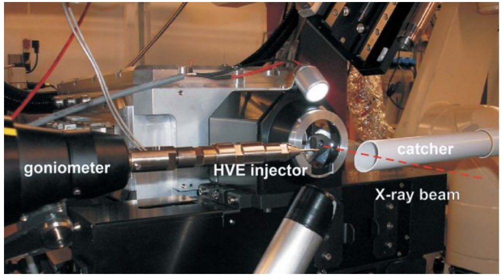


von Stetten et al, 2020

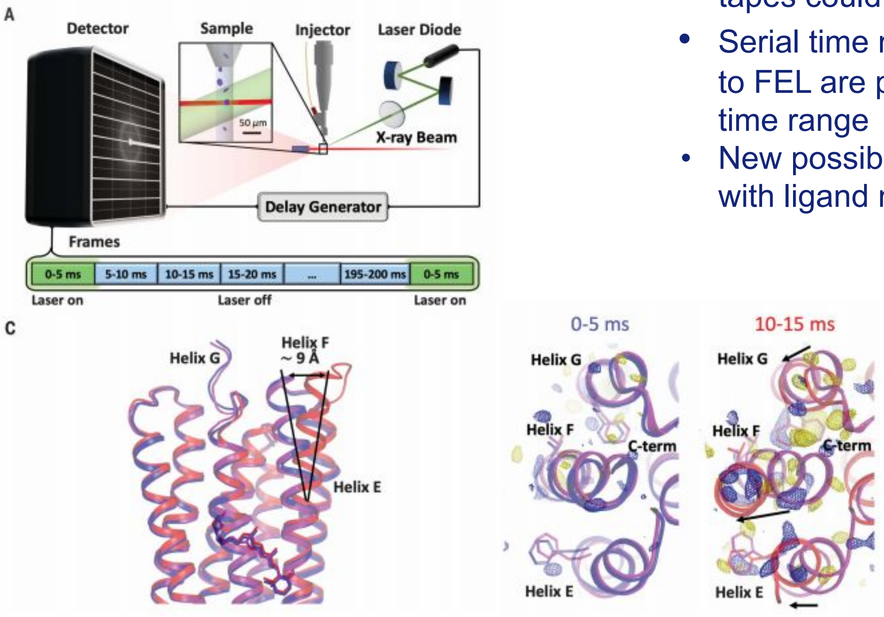


Monteiro et al 2020

- Most of “slow” sample delivery systems could be adapted to perform experiments at 3rd generation synchrotrons
- New ones such as microfluidic and tapes could be developed
- Serial time resolved experiment similar to FEL are possible, in the millisecond time range
- New possibilities for structure obtained with ligand mixing

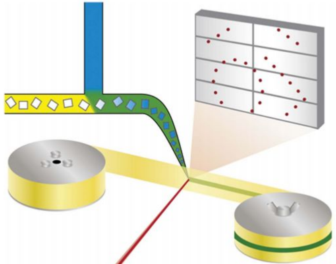


Botha et al, 2015



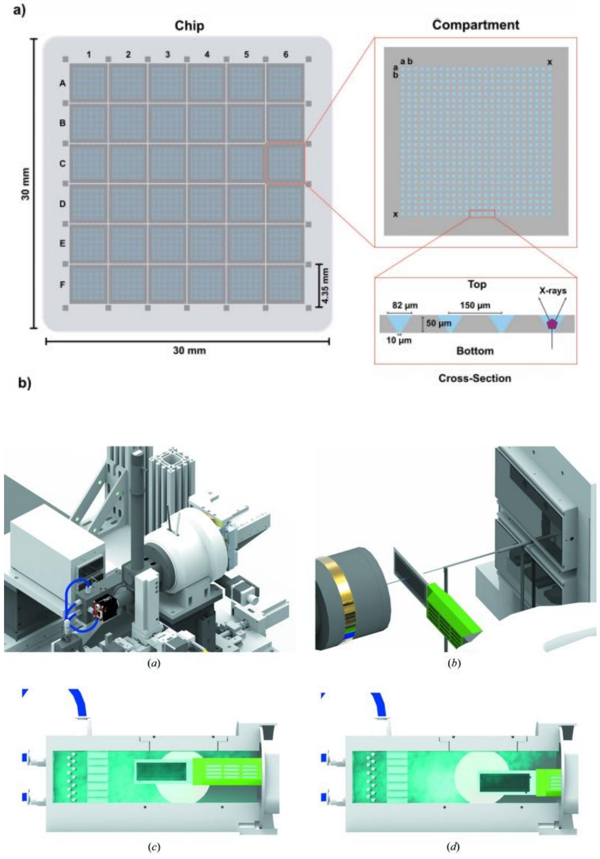
Roessler, et al 2013

Weinert et al 2019



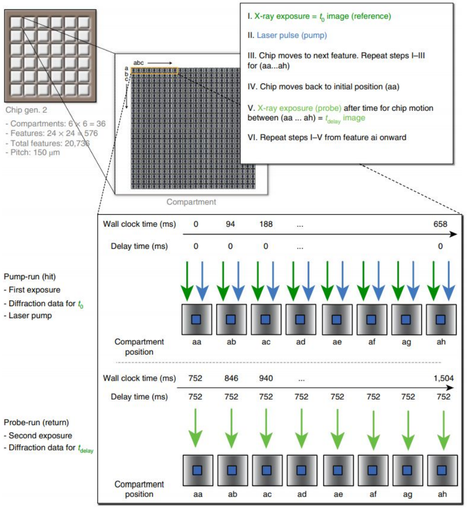
Beyerlein, et al. 2017.

FIXED TARGETS

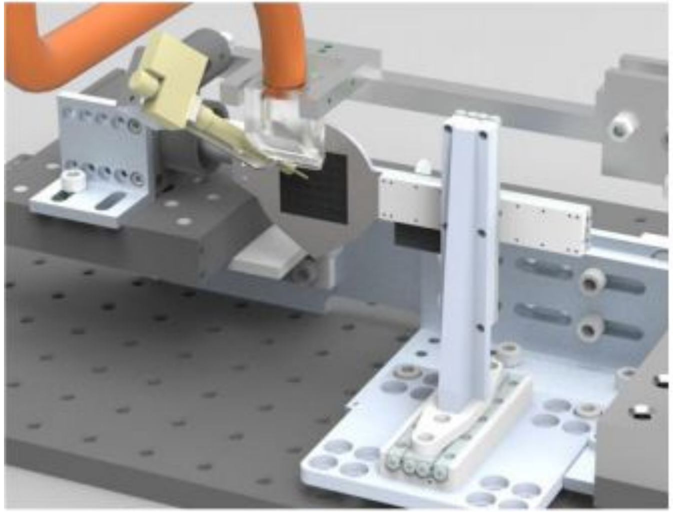


Tolstikova et al 2019

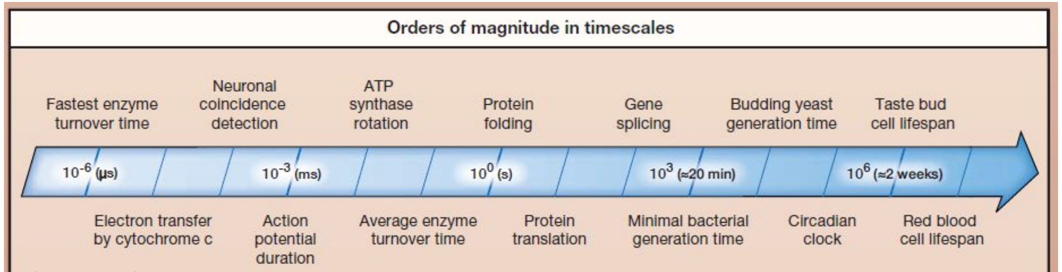
- Alternative to injectors are represented by fixed targets, where microcrystals are sitting in a regular arrays
- They can be used for light triggered time resolved experiments
- They present low background and less sample consumption
- Collect multiple time points with the Hit&Return method
- Or in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



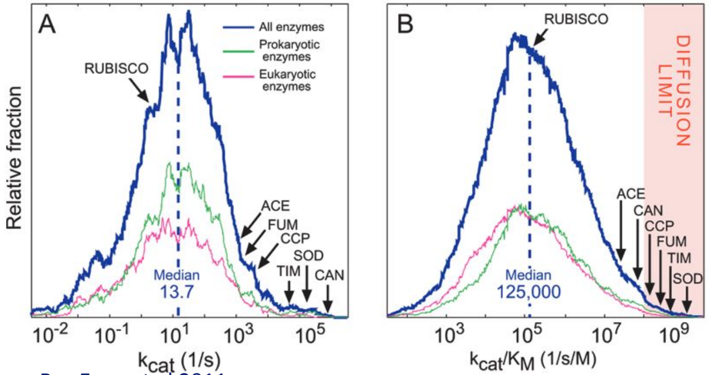
Schultz et al 2018



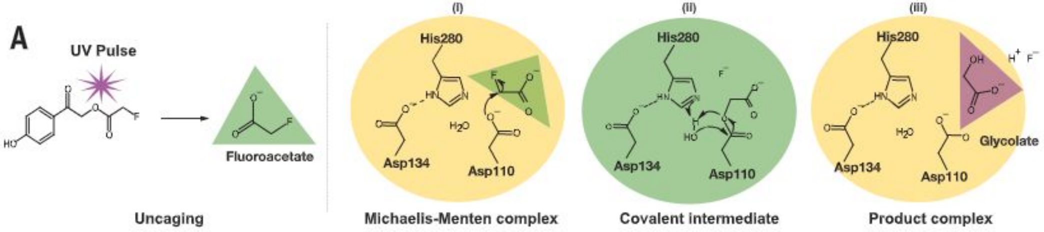
Mehrabi et al 2019



Shamir et al 2016



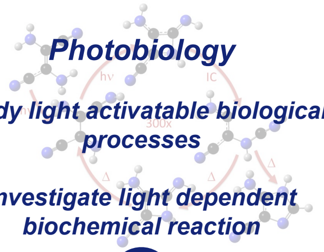
Bar-Even et al 2011



Mehrabi et al 2019

- 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
- 4th generation allow for a x10 flux and x10^5-10^6 flux density
- ID29 optical layout was designed to tackle this time resolution



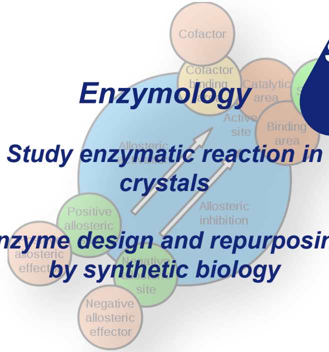
Photobiology

Study light activatable biological processes

Investigate light dependent biochemical reaction

ID29

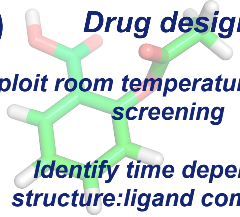
Serial and time resolved crystallography



Enzymology

Study enzymatic reaction in crystals

Enzyme design and repurposing by synthetic biology



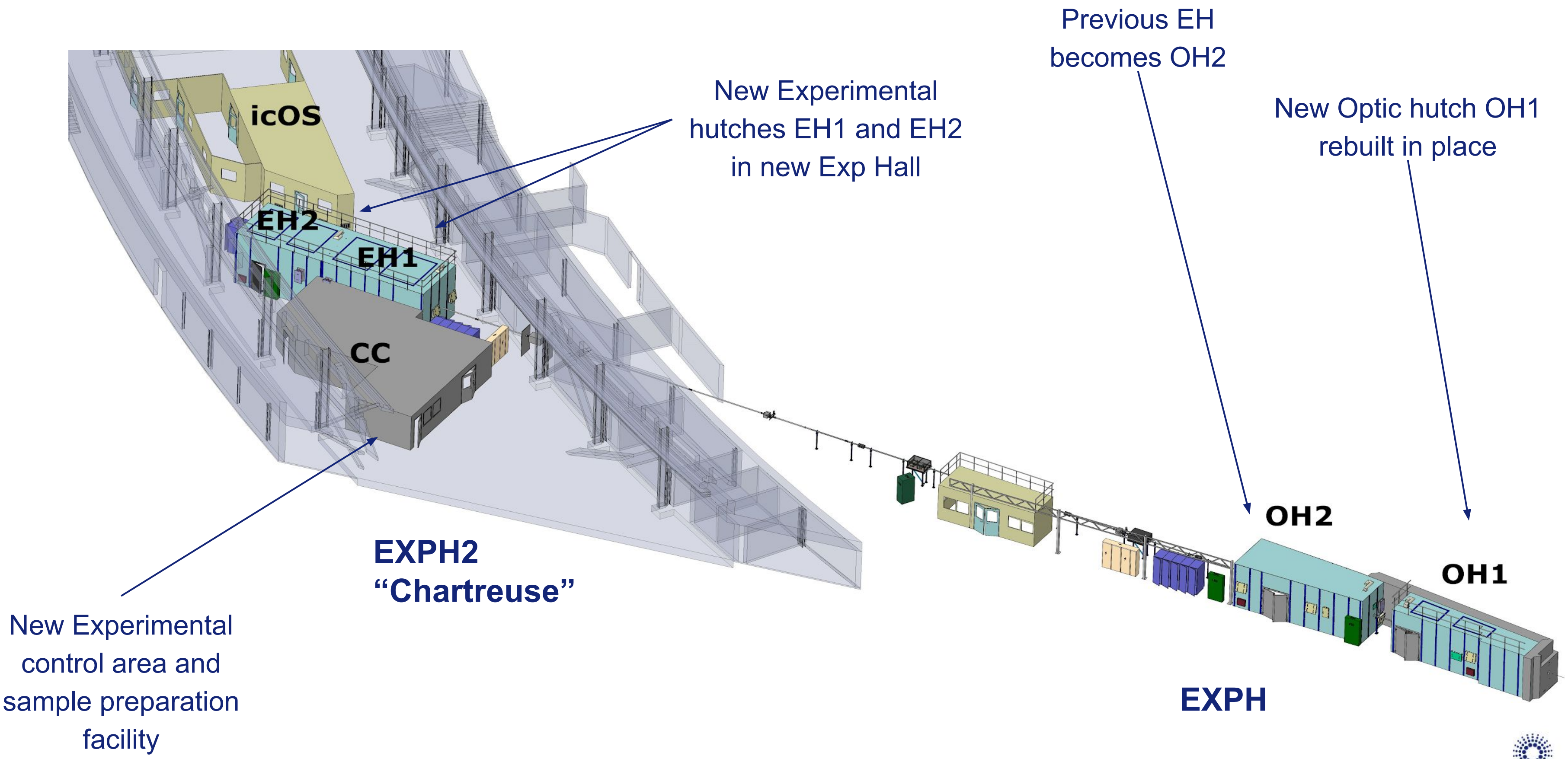
Drug design

Exploit room temperature fragment screening

Identify time dependant structure:ligand complexes

THE WISHLIST

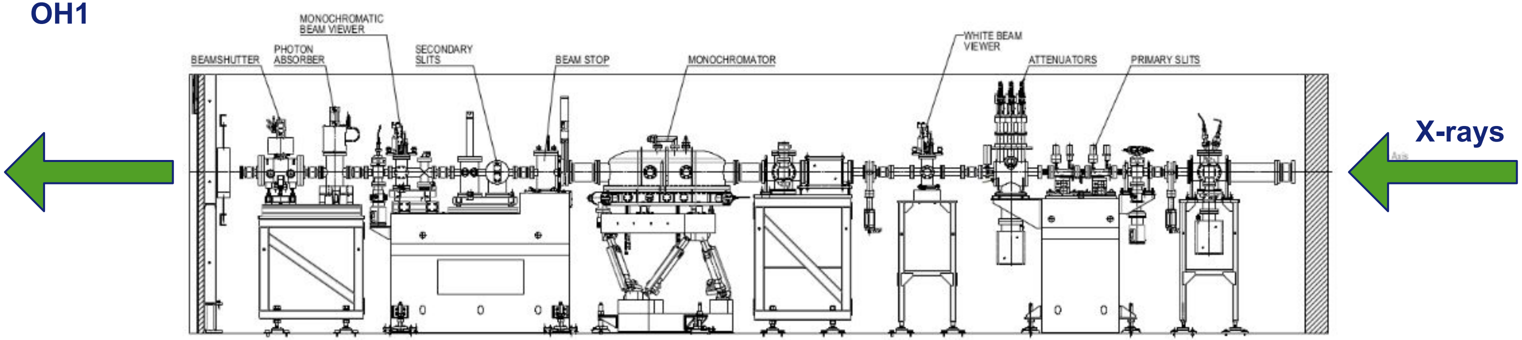
- Room temperature Serial crystallography experiment
- Extremely high flux with exposure time in μs range and high repetition rate
- Tunable over a large energy range
- Accurate control timing system to trigger events
- Adapt different sample environments and crystal delivery systems
- Low sample consumption
- A dedicated sample preparation laboratory and data analysis area



icOS
EH2
EH1
CC
New Experimental
hutches EH1 and EH2
in new Exp Hall
Previous EH
becomes OH2
New Optic hutch OH1
rebuilt in place
OH2
OH1
EXPH2
“Chartreuse”
EXPH
New Experimental
control area and
sample preparation
facility

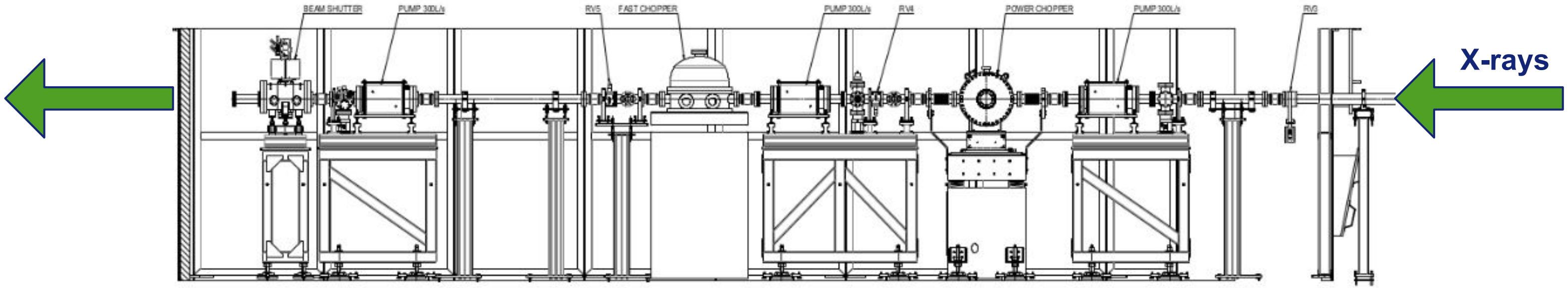
OPTIC HUTCHES

OH1



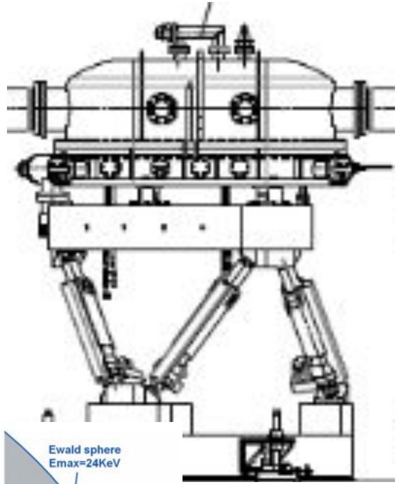
ML Monochromator

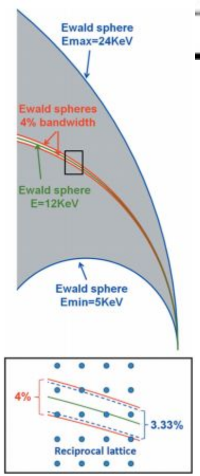
OH2

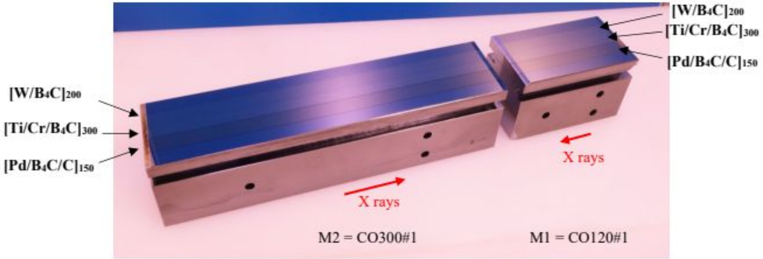


Fast Chopper **Heat load Chopper**

- A multilayer monochromator system was designed to increase bandwidth (more 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

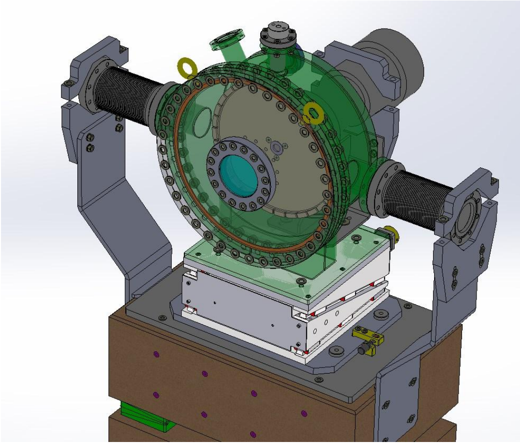


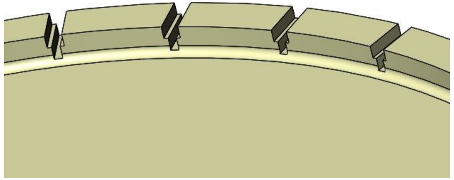




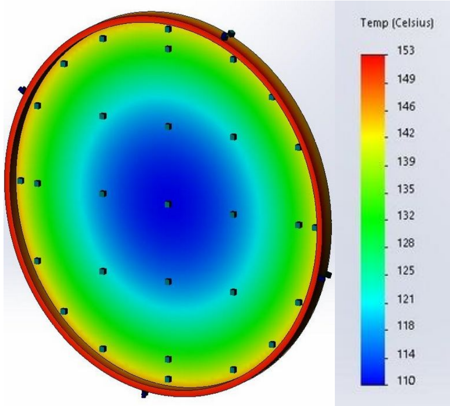
Heat load chopper

Fast chopper

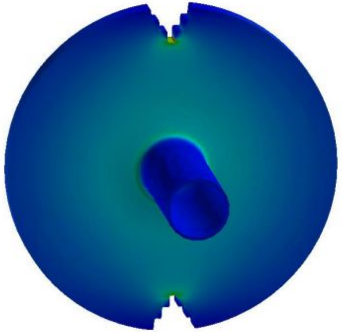


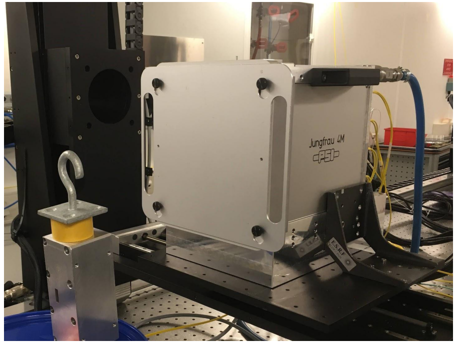


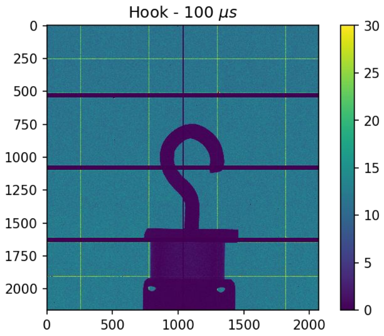
- A double chopper system removes heat load and defines short exposures
- Both choppers have variable exposure time to adapt to SR filling modes
- Heat load chopper absorbs 30 W
- Fast chopper defines short exposure time

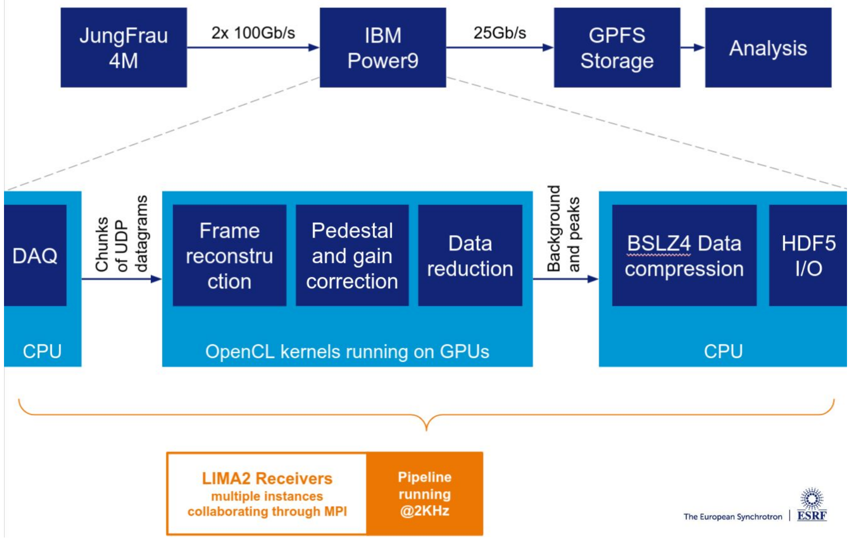


	Exposure time (µs)
1 mm	9.45
3 mm	21.10
5 mm	31.04

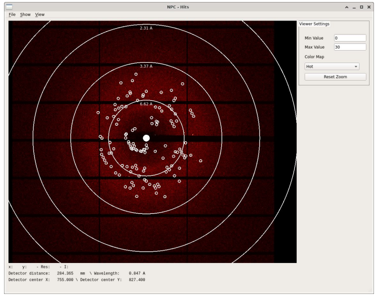


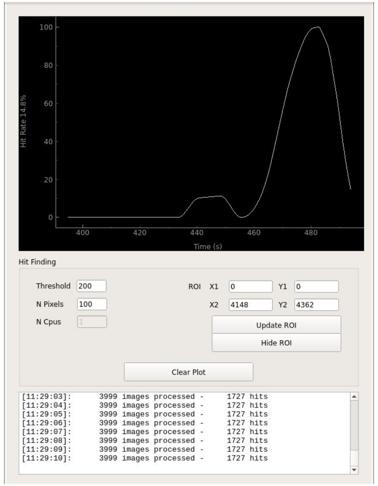


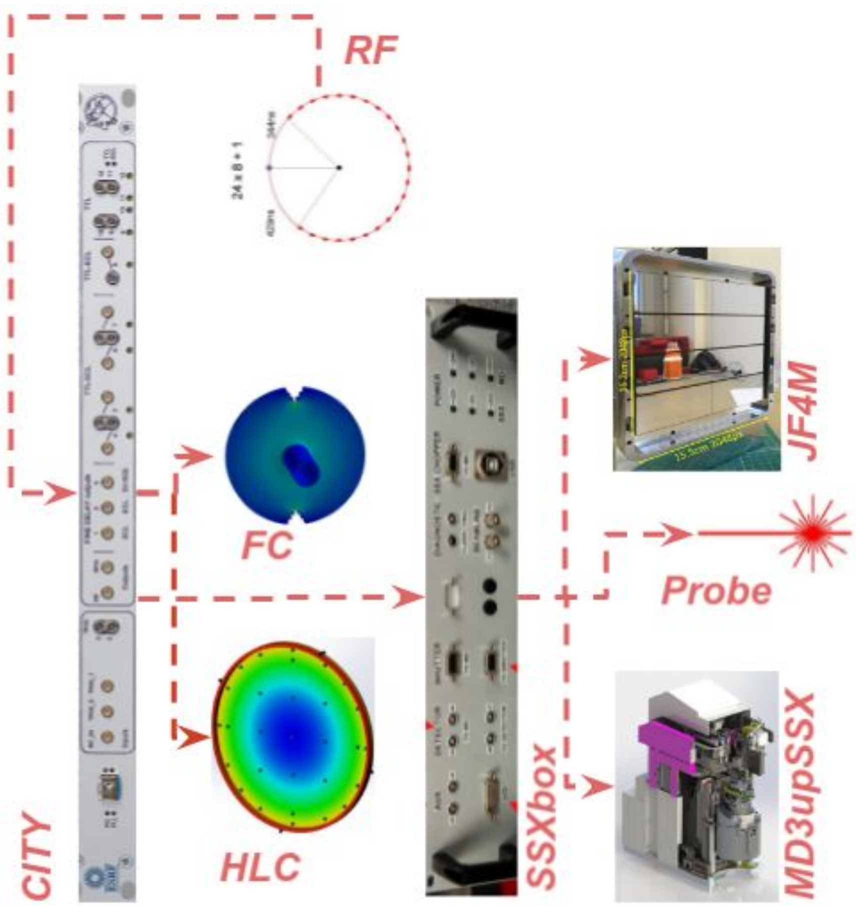


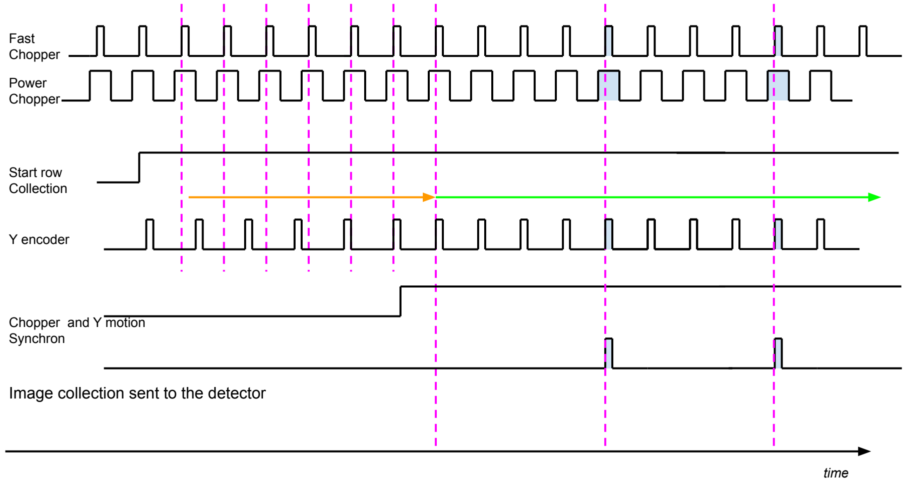


- A new version of the LImA is being developed to control and acquire data from multipanel detector
- New compression algorithms are being developed and tested to ensure data transfer
- NanoPeakCell combined with peakfinder tools for live feedback during acquisition

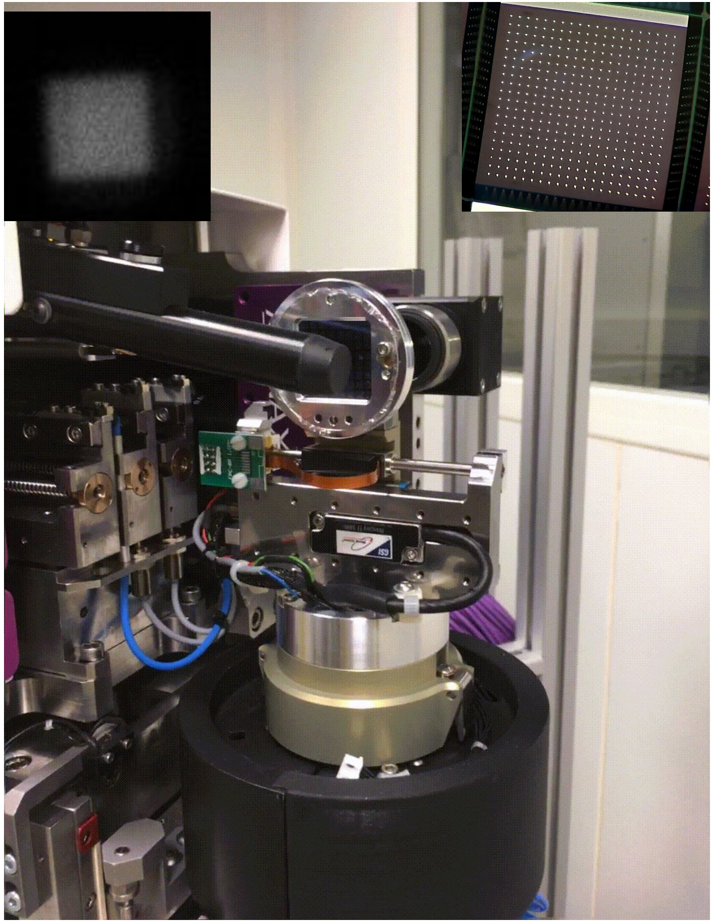


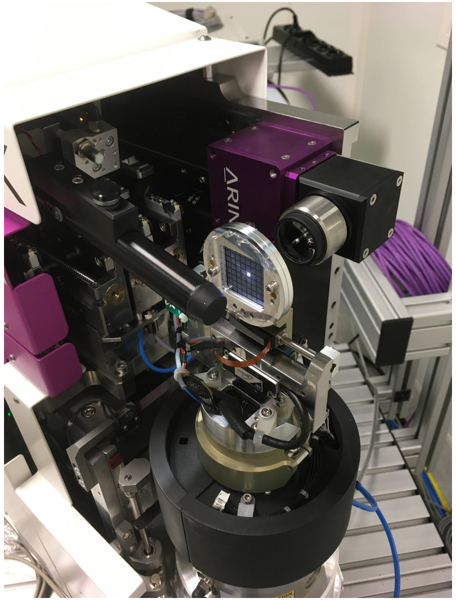




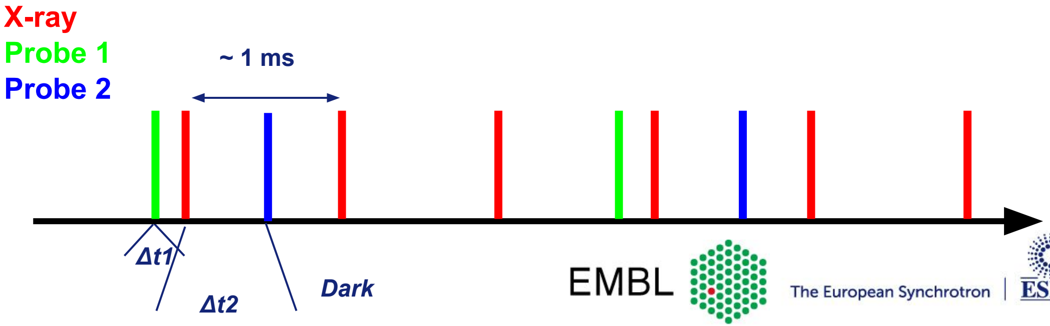


- A new developed timing system synchronises every step of the acquisition with the radiofrequency of the storage ring
- CITY and SSXbox are the two hearts of the system
- Heatload and Fast chopper are synchronised
- SSXbox propagate the clock to the data acquisition devices, MD3upSSX, X-ray detector and additional triggerings

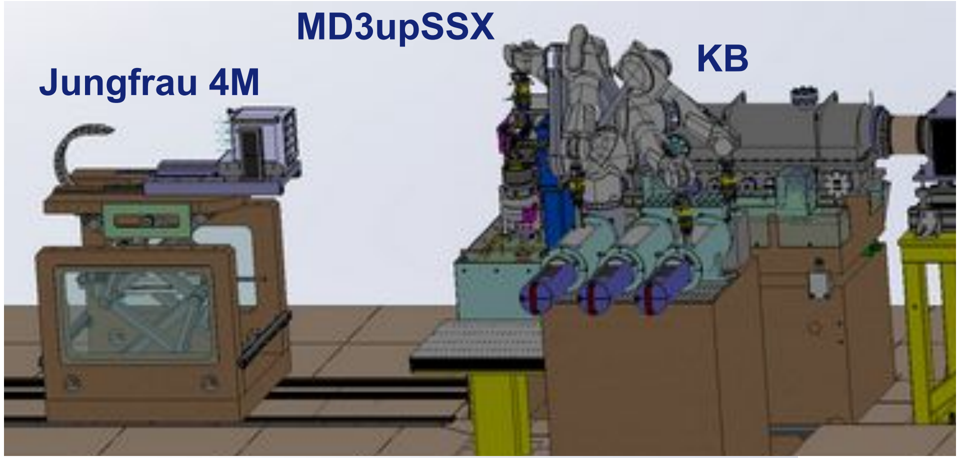




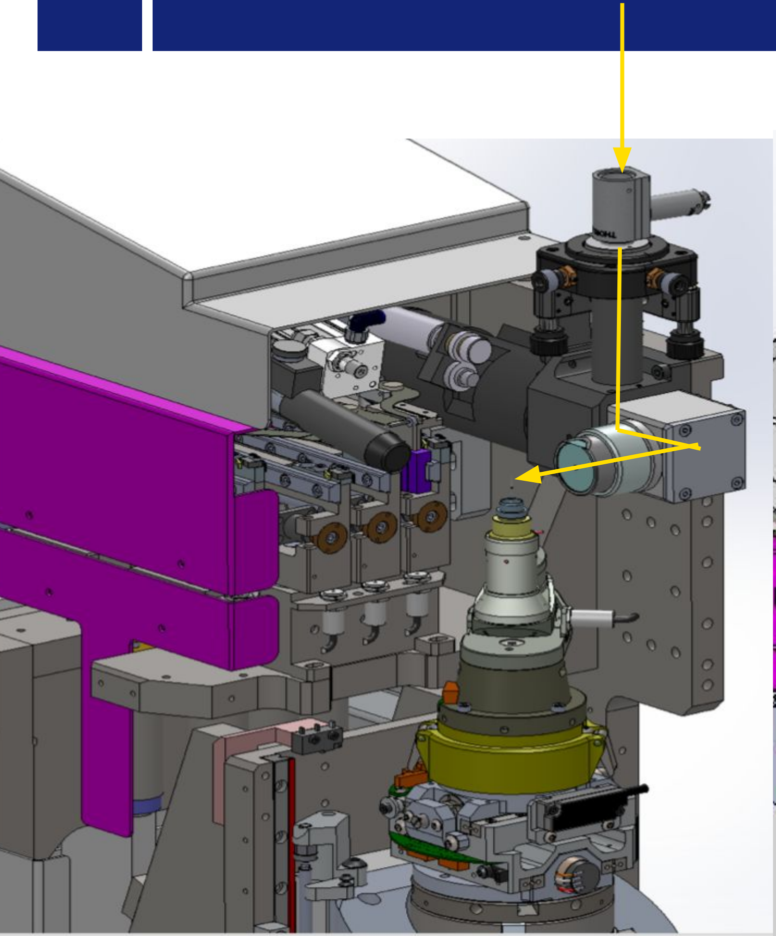
- MD3upSSX integrates a specifically developed fast translating table
- Designed for enhanced fixed target, can accommodate other sample delivery methods

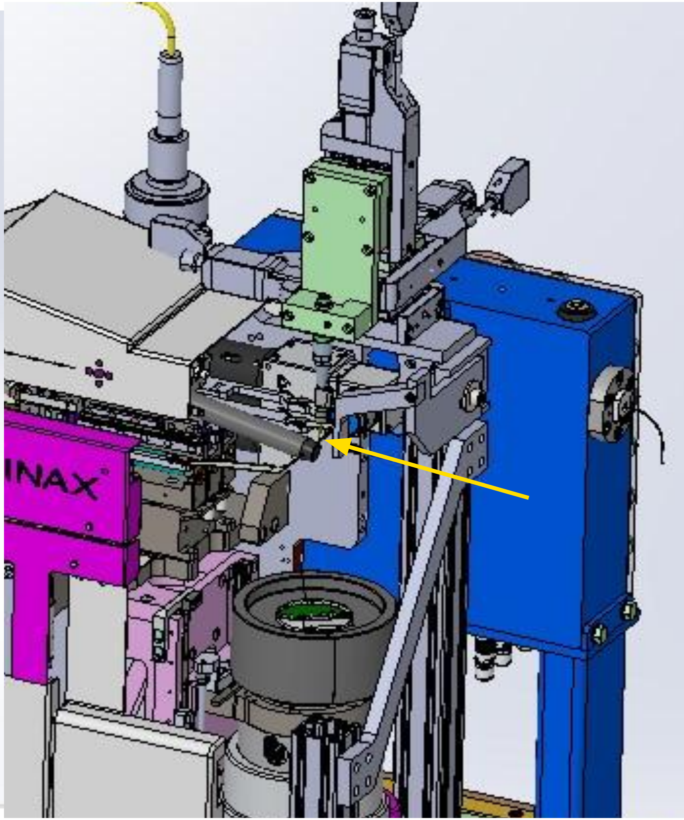


EMBL The European Synchrotron | ESRF

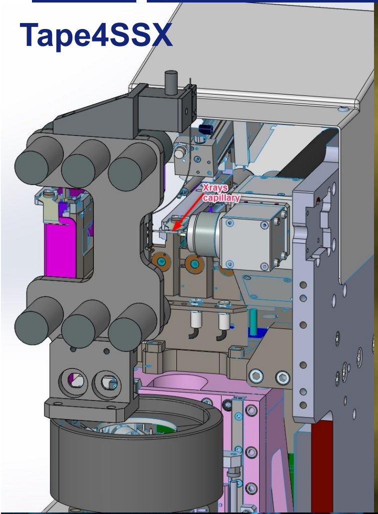


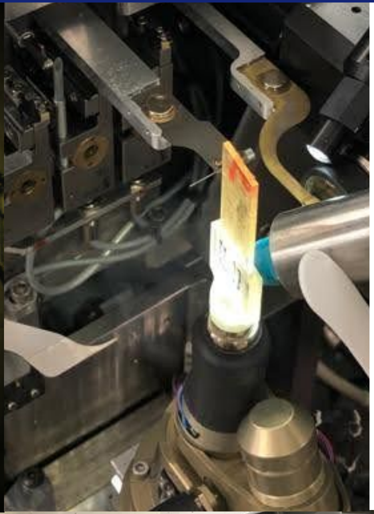
- The experimental hutch is being installed
- All components are received and being commissioned offline
- Jungfrau 4M is installed on “elevator” to deliver beam in EH2
- KB system characterised
- A new robotic system to mount/unmount fixed target support
- Storage units in humidity controlled environment



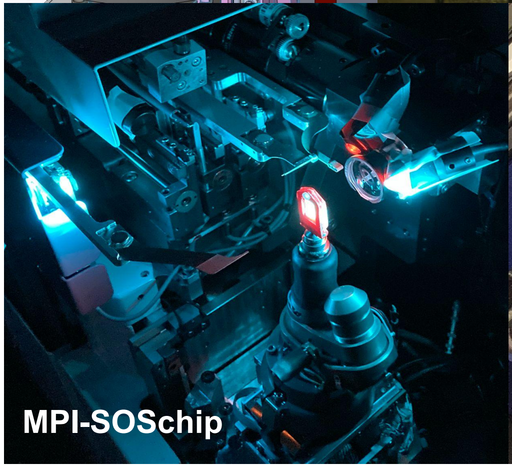


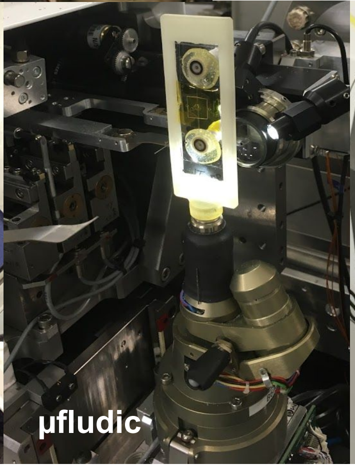
- Two possible ways to shine laser on samples are being developed: through OAV and sideway
- Laser to be delivered in June:
  - up to 500 hz repetition rate
  - 400-2000 nm tunability + 355 nm
  - < 10 mJ power

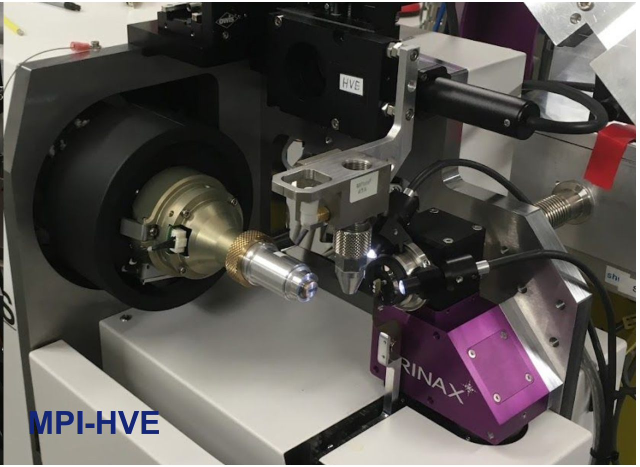


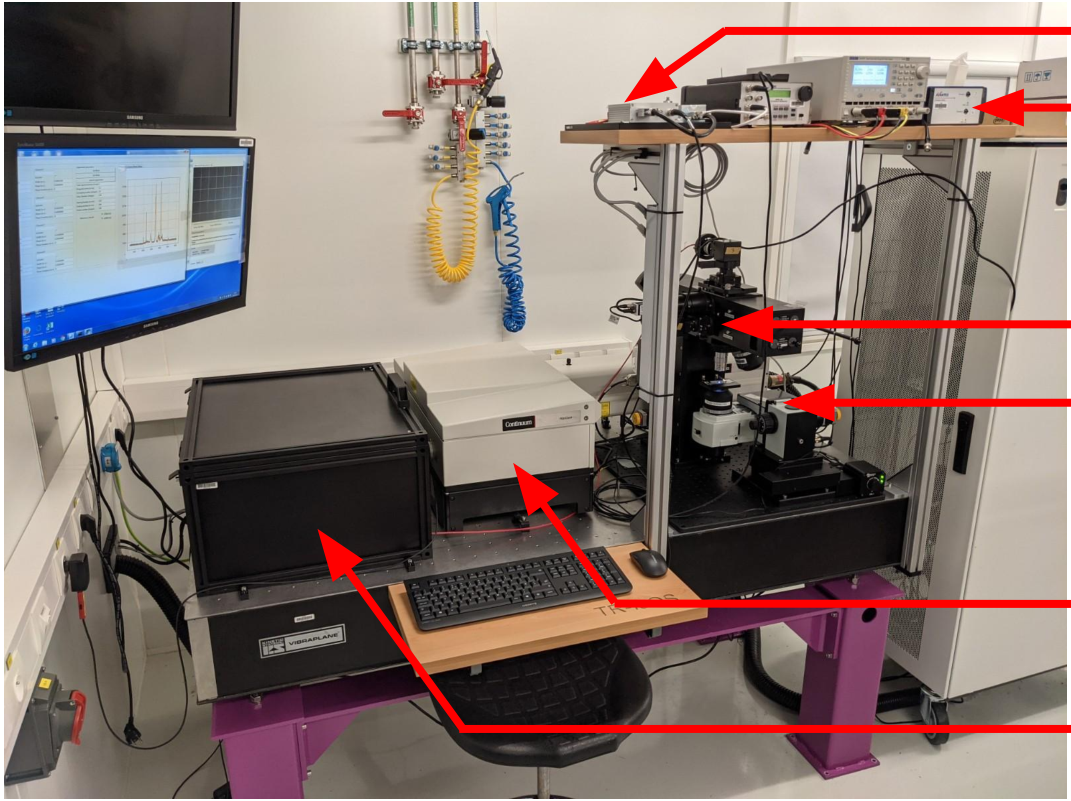


- Different projects on sample delivery are established with other groups
- Coordinated within the SSX BAG
- Tape4SSX (TU Lubeck)
- SOSchip and HVE (MPI)
- SerialX (Gothenburg University)
- Microfludic (IBS-UGA and PSCM-ESRF)









Xenon flash-lamp (2 µs pulse probe)

2-entry spectrophotometer (transmitted + reference signals)

Opto-mechanical setup with focusing objectives

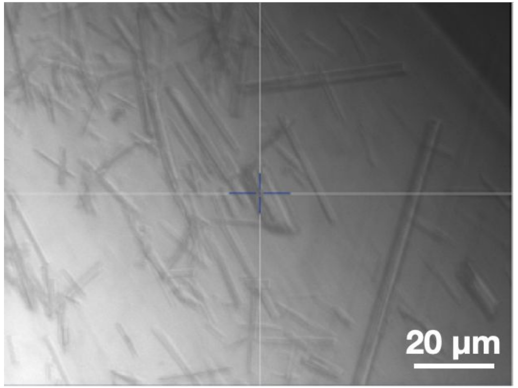
Sample holder (microcrystals between thin UV-transparent plastic sheets)

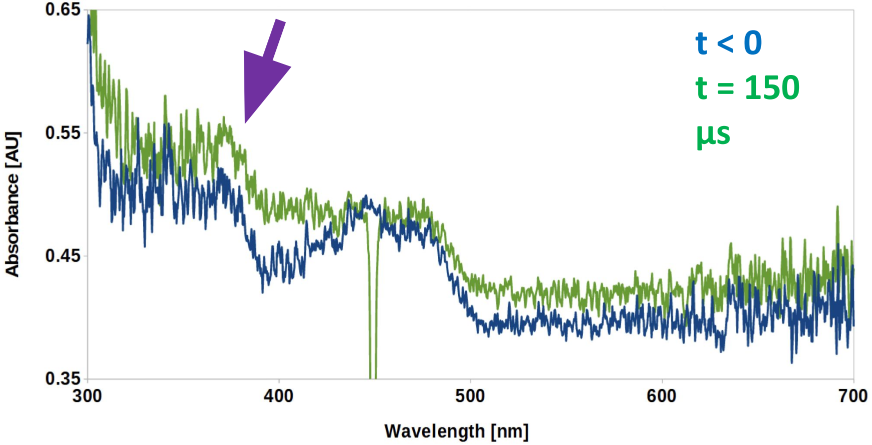
Nanosecond tunable laser (2-5 ns pulse pump)

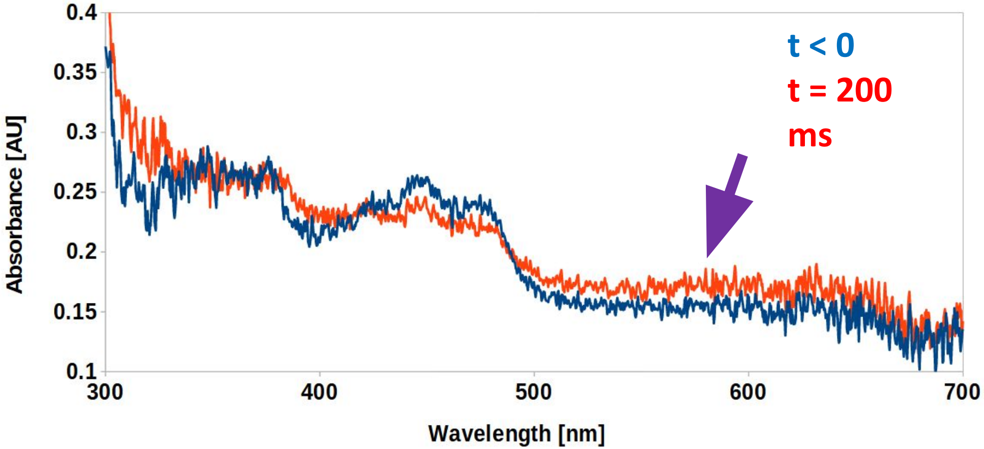
Laser signal mixing box (355 nm + [410 nm – 2000 nm] + CW (various wavelengths)

TR-*ic*OS experiments are based on the pump-probe principle in which the pump signal is provided by a nanosecond pulse from a tunable laser [355 nm, 410 nm – 2000 nm] and the probe signal by the microsecond pulse of a xenon flash-lamp [250 nm – 700 nm] allowing for the measurement of transient UV-vis absorption spectra

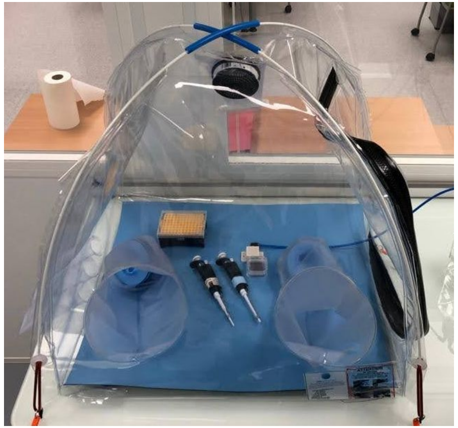
- Courtesy of Manuel Maestre Reyna (Academia Sinica, Taipei, Taiwan)
- Flavoenzyme, whose flavin goes from **oxidised** to **negatively-charged semiquinone** to **neutral semiquinone** to **reduced** during photoreaction
- Question: when does the semiquinone get protonated?



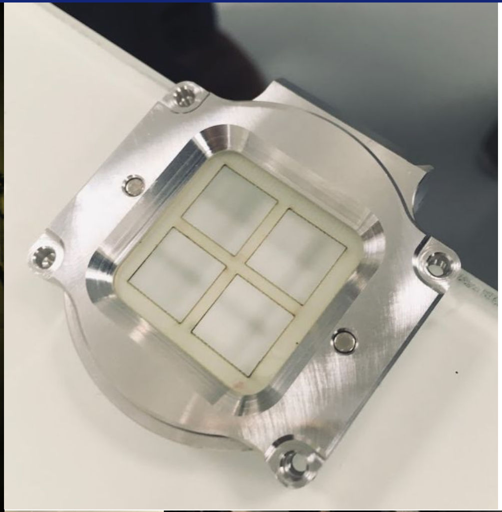




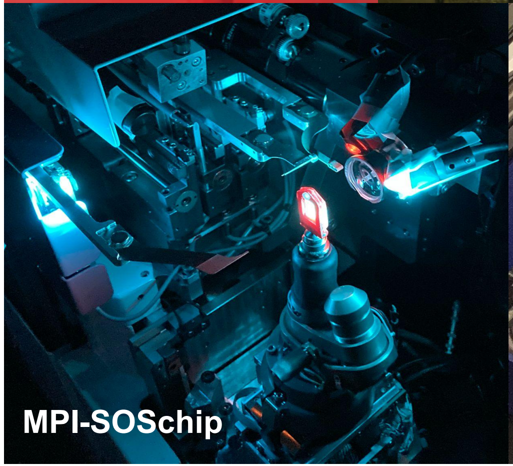
 protonation event between 150 µs and 200 ms (precise transition time still to be refined)

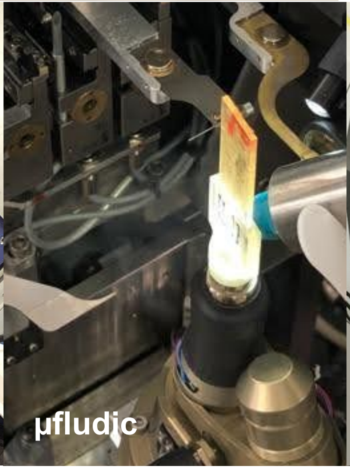


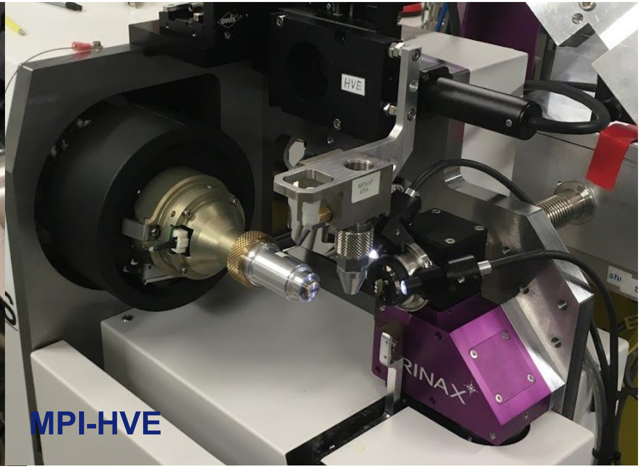
- Sample preparation laboratory
- Preliminary test of sample offline now possible on the injector bench
- Humidity controlled sample preparation station
- Fixed target loading station
- Support beginners and curious users to prepare SSX experiments
- Contact us if you want to run test with us



- Different projects on sample delivery are established with other groups
- SiChip support for MD3upSSX
- Coordinated within the SSX BAG
- SOSchip and HVE (MPI)
- SerialX (Gothenburg University)
- Microfludic (PSCM-ESRF)







Beamtime Applications

ID29 BAGs

- No minimal number of groups
- Not included in regular MX
- 2 deadline per year (BAG)
- Recurring experiments
- Multiple sessions allocated

→

- 18 shifts x session (initially)
- 1-3 shits for sample testing
- Offline (lab) experiments

- We contacted potential friendly and BAG users, we are happy to assist to cluster groups
- Both methodological and biological projects are welcome
- Contact us anytime to be friendly users, discuss feasibility or for any question

ID29 Rolling

- Call permanently open
- Beamtime in a short time
- Less regular users
- Single experiment (on few sessions)

→

- Up to 18 shifts (based on proposal)
- 1-3 shits for sample testing
- Offline (lab) experiments

New Proposal | Proposals In progress | Proposals with Final Number/Previous Proposals | In progress, as Co-Proposer

Information for users

- The next deadline for proposals submission will be in 2020. Consult the guidelines for more details. Any proposals subm
- The submission of Rolling Proposals for CryoEm will continue during the shutdown period.
- Please consult the Information on Data Provided concerning Scientific Use of ESRF
- Before creating your proposal, make sure that each co-proposer has created his(her) account.

Submit New Proposal

To create your Beamtime Application Form, select the appropriate Proposal by clicking on the corresponding button.

- ○ Standard ESRF Proposal (for public beamtime on ESRF and CRG beamlines) [?]
- ○ Structural Biology : Rolling Proposal [?]
- ○ Structural Biology : BAG Proposal [?]
- ○ Long-Term Project [?]
- ○ CRG Proposal [?]
- ○ Industrial Experiment (Non Structural Biology) [?]
- ○ Industrial Experiment (Structural Biology: MX and BioSAXS) [?]
- ○ Inhouse proposal

Beamtime

Summary of Beamtime Requested FOR SIX MONTHS

*

☐ Multi-wavelength		shifts
☐ Single-wavelength		shifts
☐ BM29 Bio-SAXS		shifts
☐ Cryo-EM		shifts
☐ ID29 SSX & TR-SSX		shifts
Total		shifts

Requests for specific beamlines or equipment:

- Select BAG or Rolling
- Select ID29 SSX & TR-SSX specifying number of shifts
- Shifts organization (experiment, testing, offline) will be done at the scheduling

We need support from:

☐ PSCM Labs (Science Building) — If you wish to use the l can be found HERE. Th

☐ icOS Lab (in crystallo Optical Spectroscopy) — If you wish to use the i HERE.

It is recommended that you check with your local contact beforehand that ar

- Access to TR-icOS directly via A-form
- As to access other icOS facilities
- Possible also via MX A-forms

EBSL8 IN A NUTSHELL

Energy	10-20 keV + 35 keV
Bandwidth	0.3% and 1% (ΔE/E)
Flux	> 10^{15} ph/s
Beamsize	0.6 x 0.4 μm^2
Beam divergence	1.9 x 0.7 mrad
Exposure time (Fast chopper)	10 - 20 - 30 µs
Exposure time (HL chopper)	100-150 µs
Repetition rate (initial)	925 hz (x2 possible)

07/2021 — **First monochromatic beam**

Commissioning of hexapod and radiation test of OH2

10/2021 — **Installation of OH2**

Commissioning Heatload chopper

02/2022 — **Commissioning of EH1**

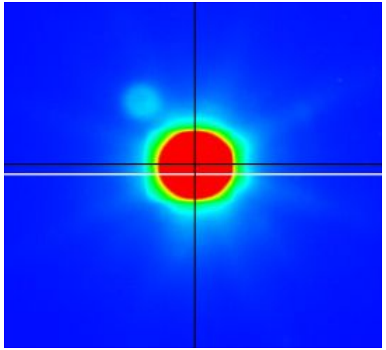
Commissioning of MD3upSSX, timing system, Jungfrau detector

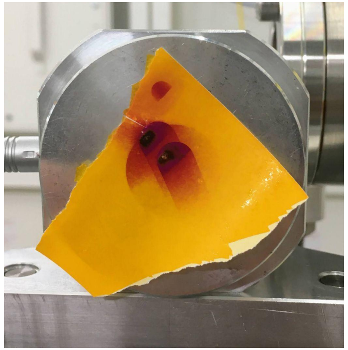
03/2022 — **BAG proposals deadline 1st of March**

06/2022 — **Friendly users**

Prepare for user mode in August 2022

09/2022 — **Beginning of user mode**





Photon Flux Estimation

	values	UNITS
I measured =	4.00E+02	mA
Energy =	12.50	KeV
Thickness of Silicon photodiode =	300	µm
Thickness of aluminum =	30	µm
Aluminum transmission =	99.67%	**(for 6 to 20 keV)**
Thickness of air =	4	cm
Air transmission =	98.84%	**(for 6 to 20 keV)**
Flux photon =	**1.046E+15**	**Number of photons**

Shibom Basu
Julien Orlans

ESRF Structural Biology Group
Hugo Caserotto
Nicolas Coquelle
Fabien Dobias
David Flot
Jonathan Gigmes
Thierry Giraud
Gordon Leonard
Christoph Mueller-Dieckmann
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Daphne Lorphevre
Carlos Muñoz Pequeño
Pascal Theveneau

ESRF Detector & Electronics Group
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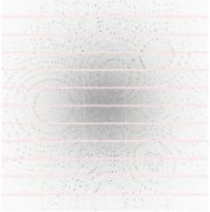
Samuel Debionne
Andy Gotz
Alejandro Homs
Jerome Kieffer
Marcus Oscarsson
Olof Svensson

ESRF Optics Group
Ray Barrett
Christian Morawe
Amparo Vivo

Follow the progress
@DdS_ID29

Configuration 3

Parameter	Value	Image (Link for full resolution)
Divergence (mrad) (HxV)	1.9 x 0.7	10 µm MaxI:502128, AvgI:2 
Flux (ph/s)	10^{15}	
Distance (mm)	250	
Beamsize (µm) (HxV)	0.6 x 0.5	
Energy (keV) - Wavelength (Å)	10 - 1.24	
Bandwidth ΔE/E (%)	1	5 µm MaxI:62671, AvgI:0 
Crystal size (µm)	10 - 5	
Exposure time (s)	10^{-6}	
Sample thickness (mm)	0.01	
		5 µm - naked MaxI:62563, AvgI:0