Pierre Aller, Matthew Rodrigues and Abbey Telfer
XFEL-Hub @ Diamond light source
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XFEL-Hub & time-resolved serial crystallography

~~17th September 2025~~

Overview:

- ❑ XFEL-Hub introduction
- ❑ X-ray sources: Synchrotron and XFEL
- ❑ Sample deliveries current and upcoming
    - High viscosity extruder
    - Fixed target
    - Hybrid method: drop on tape

- ❑ **XFEL-Hub introduction**
- ❑ X-ray sources: Synchrotron and XFEL
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XFEL Hub

Allen Orville, Principal Scientist XFEL-Hub
Pierre Aller, Senior Beamline Scientist
Matthew Rodrigues, Beamline Scientist
Abbey Telfer, PDRA
Tiankun Zhou, PDRA
Emily Freeman, PhD student
Jack Stubbs, PhD student
Eloisa Wheatley, PhD student
Madeleine Smith, year in industry student

http://www.diamond.ac.uk/Beamlines/Mx/XFEL-Hub.html

XFEL-Hub contact

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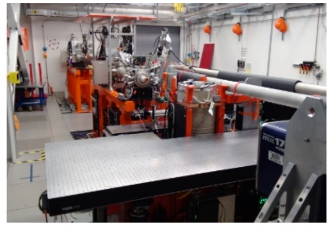
Instruments by Science Group

Macromolecular Crystallography | Soft Condensed Matter | Imaging and Microscopy | Biological Cryo-Imaging | Magnetic Materials | Structures and Surfaces | Crystallography | Spectroscopy

Welcome to the XFEL Hub at Diamond

The UK is taking a leading role in the development of a new structural biology facility (SFX) at the European X-ray Free Electron Laser (XFEL), in Hamburg, Germany, and a complementary facility at Diamond (The UK XFEL Hub) to help develop the required expertise.

The UK is a leading member of the serial femtosecond crystallography user consortium (SFX-UC), and through its role in the collaboration will deliver a dual instrument at the European XFEL that will enable visiting scientific users to image macromolecules from nanocrystals on the SFX instrument and single particles using scattering of coherent X-rays and detecting the coherent diffraction pattern on the SPB instrument.



New MFX end station at LCLS, SLAC National Accelerator Laboratory, USA

Serial femtosecond crystallography (SFX) was the term coined to describe the protocols used to collect the first diffraction data from nanocrystals at the LCLS in Stanford, USA. Scientists there were able to exploit the ultrashort pulses generated by LCLS to collect single shot diffraction images from nanocrystals. Diffraction from such small crystals was made possible due to the high brilliance of the beam (many orders of magnitude more than achievable at a synchrotron).

The SFX-UC brings together members of the scientific communities in Germany, United Kingdom, Sweden, Slovakia, Switzerland, United States and Australia. The project is being managed in Hamburg by Adrian Mancuso. The UK structural biology community is represented by Jim Naismith (lead PI, St. Andrews), Jan Lowe (LMB, Cambridge), Henry Chapman (CFEL, Hamburg), Martin Walsh (Diamond) and Dave Stuart (Oxford & Diamond).

At Diamond Dr Allen M. Orville has been appointed group leader of the XFEL Hub which will act as a focus for a number of activities:

- Development of hardware and software for SFX
- Provision of a sample environments lab to enable users to prepare samples for current liquid jet technologies at operating hard X-ray FELS
- Provision of a user access program for SFX/SPB and currently operating hard X-ray FEL facilities worldwide.

Housed within the existing Diamond infrastructure, the hub will enable users to fully prepare for their experiments with currently operating XFELs and the European XFEL when it comes online in Hamburg in 2017. The UK Hub will provide support in terms of sample preparation, data processing and training. There will also be a dedicated fibre link from Hamburg to Harwell enabling users to carry out data analysis back in the UK, with support from the UK Hub team.

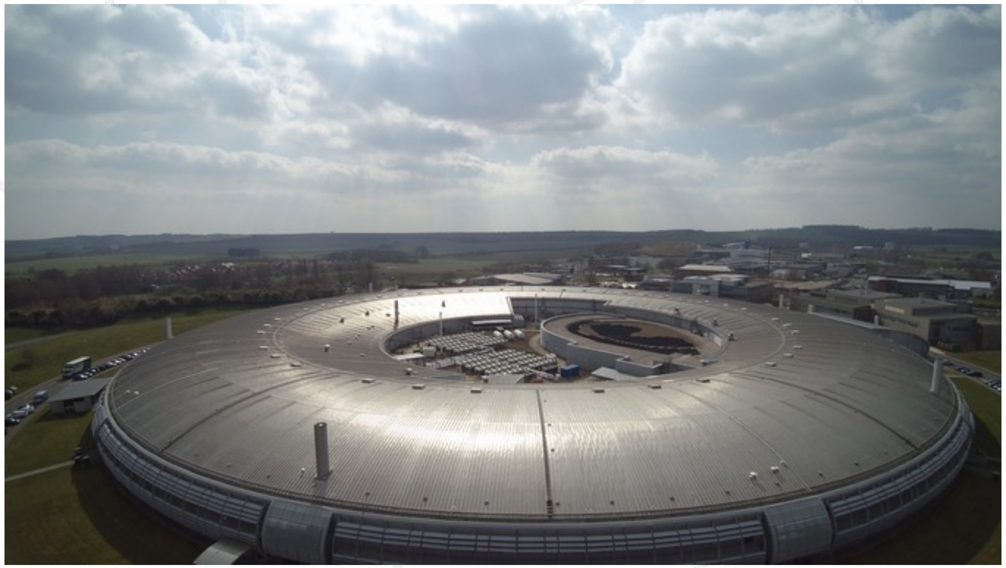
For more information on the UK-XFEL Hub, please contact Allen Orville.

More about SFX and the European XFEL see latest news.

XFEL-Hub at Diamond

Travel grant for XFEL experiment:

- Only UK based researchers
- Up to 4 members on the same experiment can apply
- Non-proprietary XFEL beamtime
- Only flight ticket will be reimbursed



Dynamic Structural Biology BAG

- Open to anyone interested in SSX
- ~ 10 shifts available on I24 /Allocation period
- Sample delivery systems available: Fixed target and LCP extruder

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Synchrotron facilities

emittance: measure of size and angular spread of the e^- beam

- 1st Generation sources
  - parasitic
- 2nd Generation sources (80's)
  - dedicated machines to synchrotron rad. emittance typically high (150nmrad)
- 3rd Generation sources (90's-00's)
  - low beam emittance, use of insertion devices (wigglers and undulators)–emittance on order of 3-20nmrad
- 4th Generation sources (20's)
  - Change the lattice, lower beam emittance ~ 130 pmrad

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Image from Canadian Light Source

XFEL sources

Linear accelerator followed by undulators

- 700-1.1km (Sacla, PalXFEL, SwissFEL)
- 3km LCLS-II
- 3.4km European XFEL

European XFEL

SACLA

XFEL facilities

5 XFEL facilities:

- **LCLS-II** (USA)
- **SACLA** (Japan)
- **PAL XFEL** (Korea)
- **SwissFEL** (Switzerland)
- **European XFEL** (Germany)

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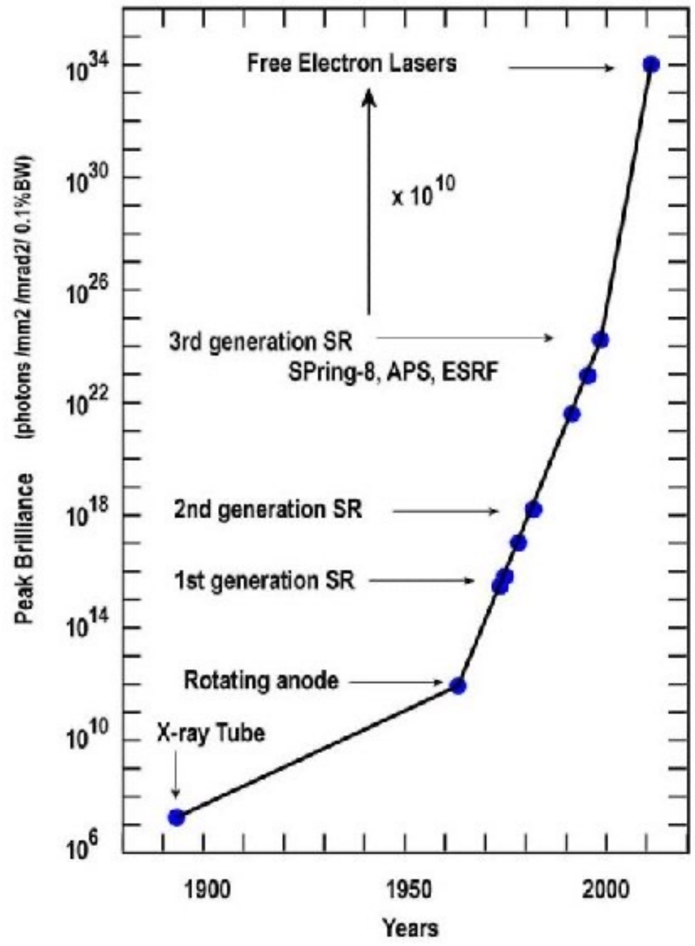
Synchrotron vs XFEL: Beam properties

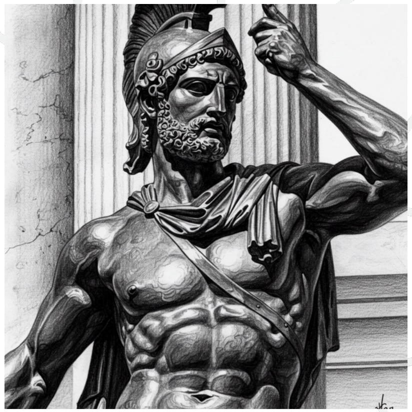
XFEL	Synchrotron
fs pulse	Continuous (ish)
Coherent beam	Incoherent beam
Limited access	Easy access

~10^{10} more brilliant

Photons in 1 XFEL pulse ≃ 1s of synchrotron beam



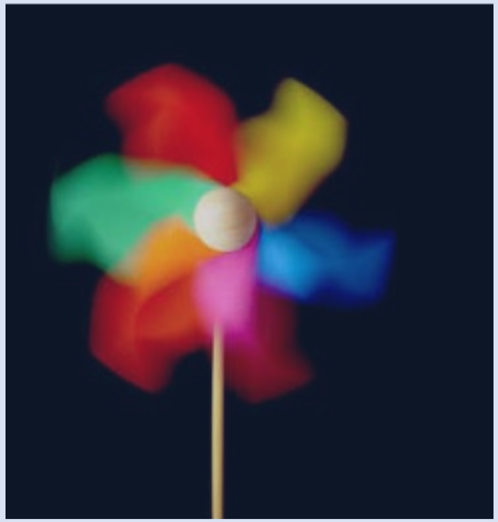
From Shintake T., Proceedings of PAC07, Albuquerque, New Mexico, USA



Motionless object

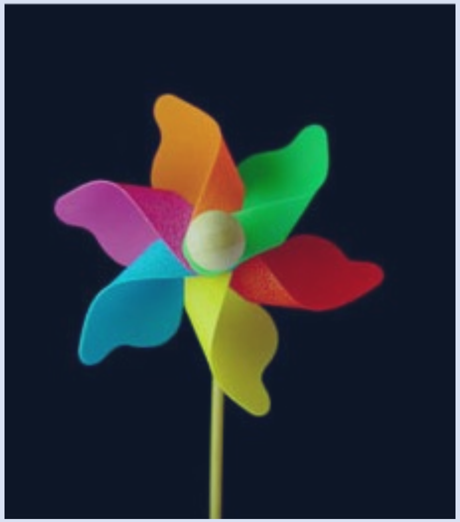
Synchrotron

Slow shutter speed



Faster shutter speed

XFEL



Shutter always open
Very short flash of light

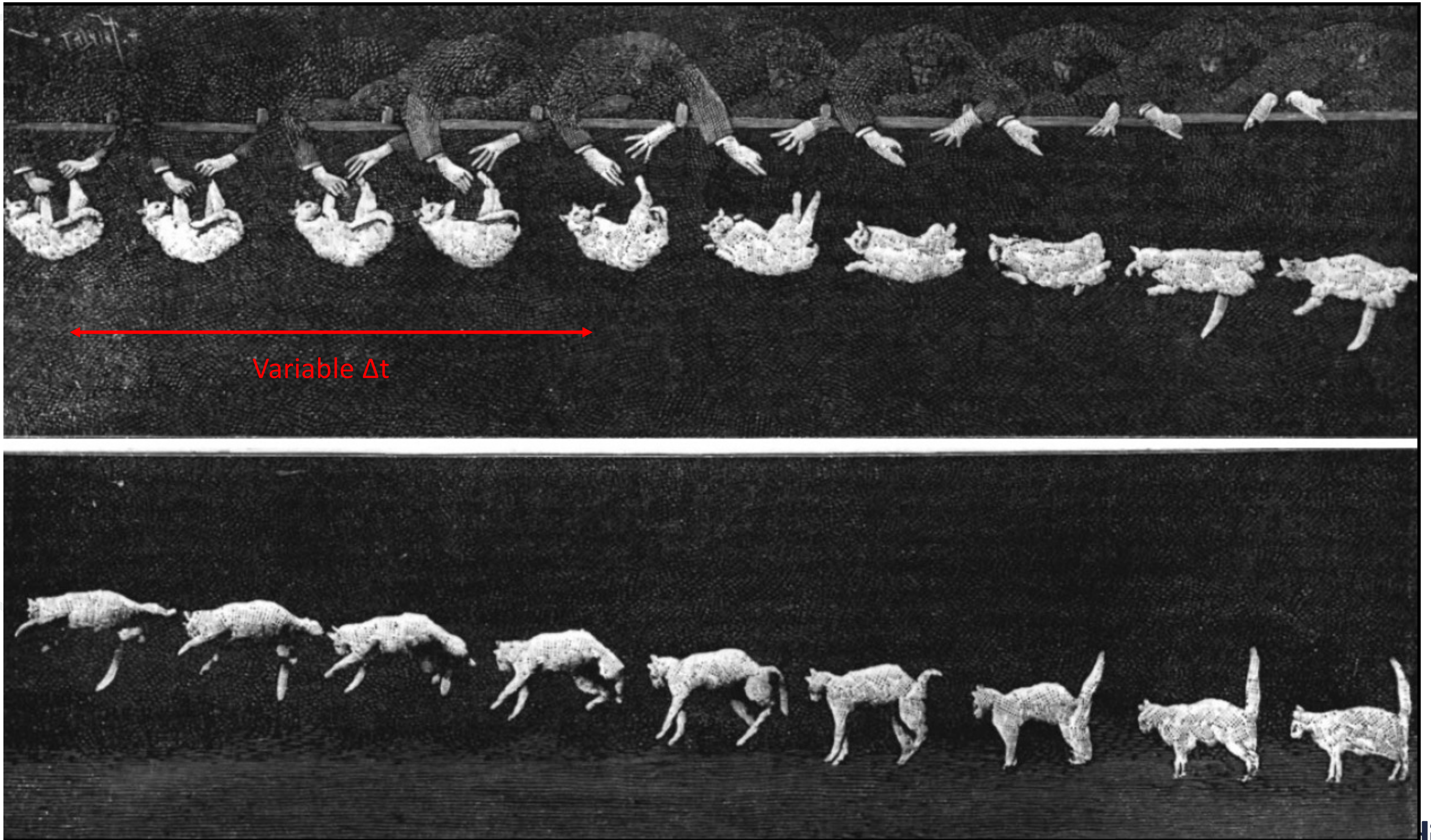
=> Better temporal definition

Dynamic structural biology

Intermediate states

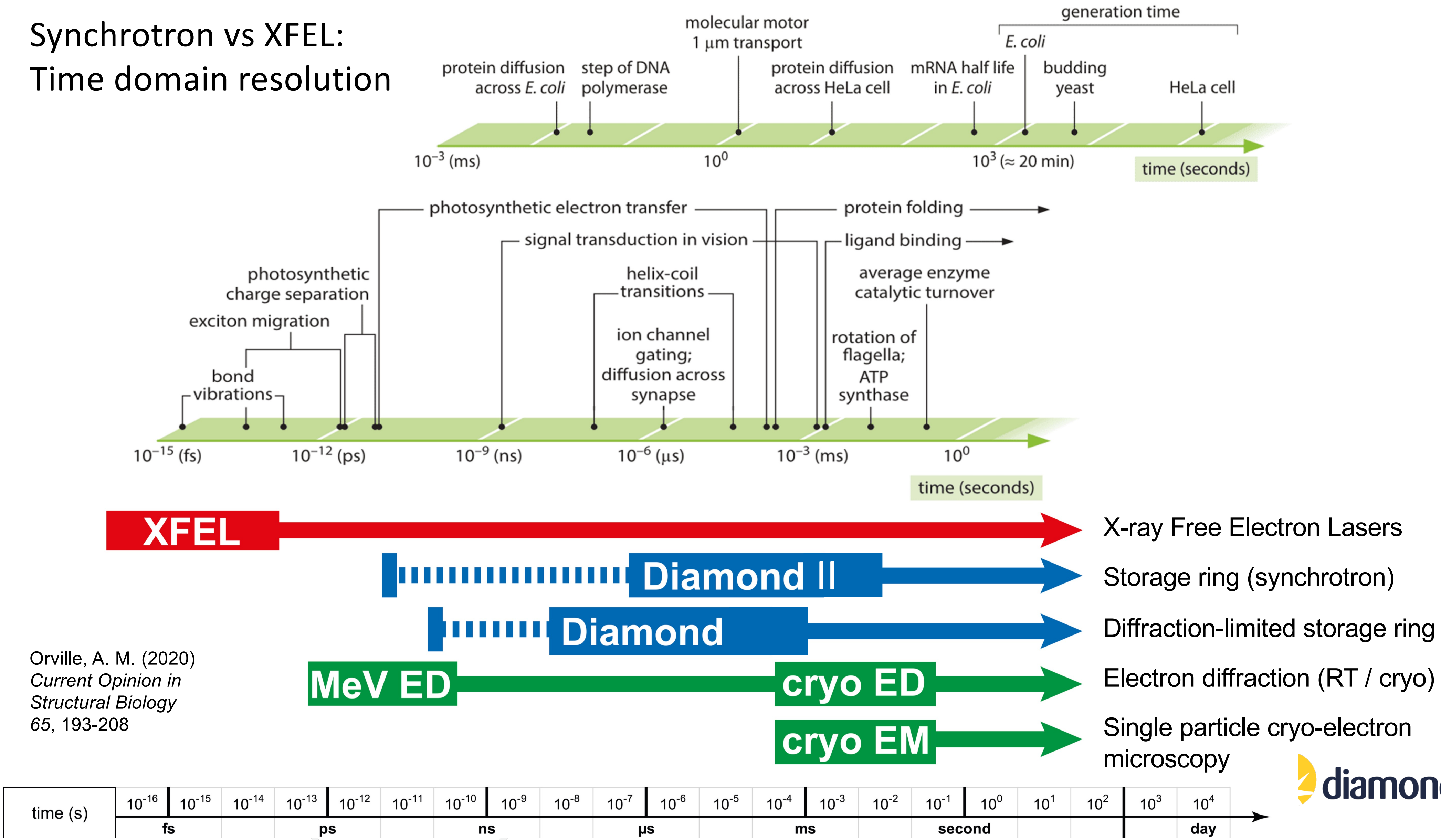
Resting state at
t=0

Final state



Falling Cat, 1894 by Etienne-Jules Marey

Synchrotron vs XFEL: Time domain resolution



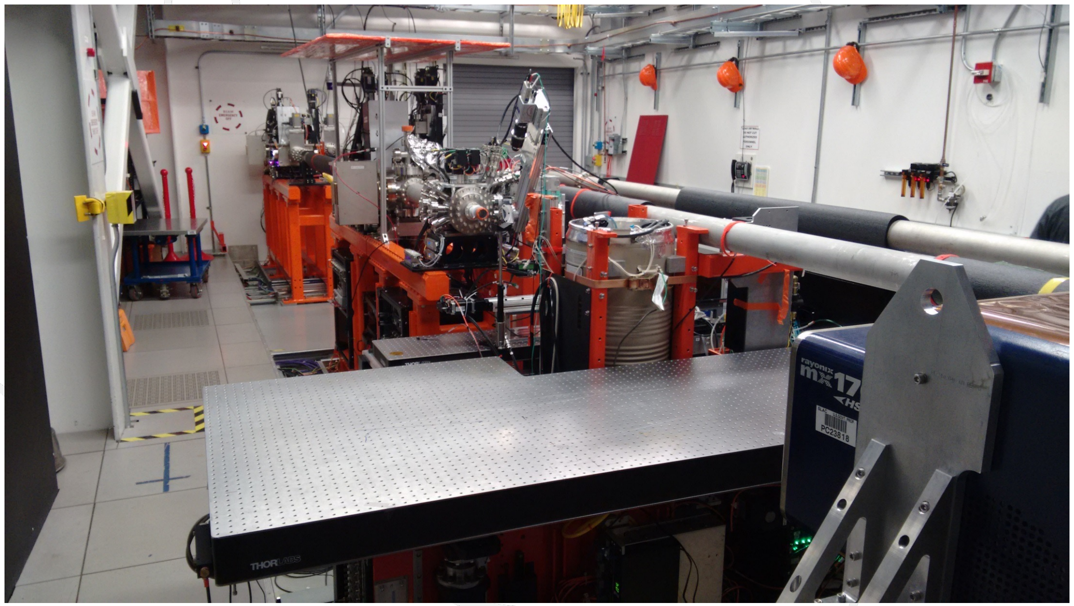
Orville, A. M. (2020)
Current Opinion in Structural Biology
65, 193-208

Endstation

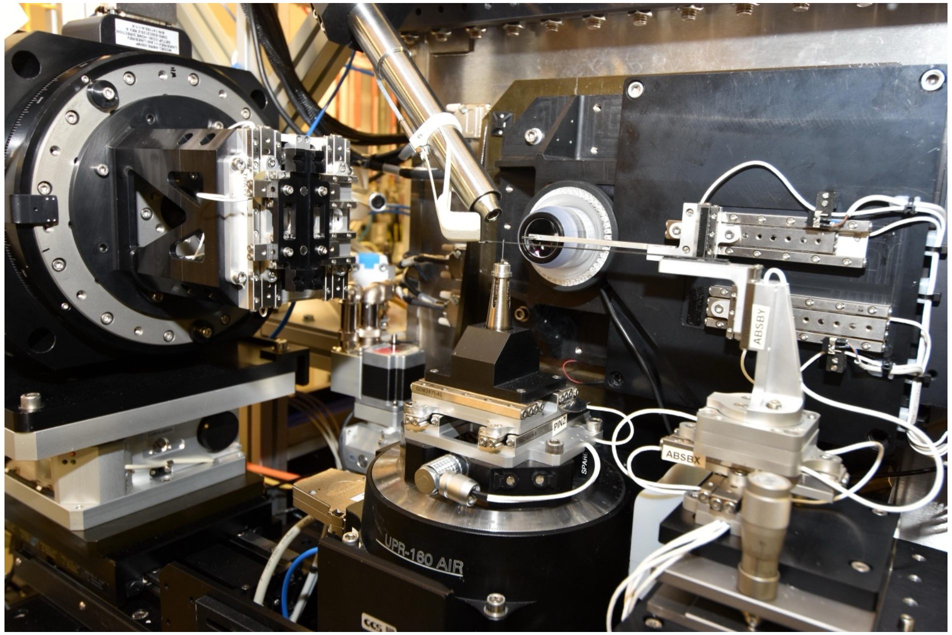
XFEL

Synchrotron

User friendly



MFX experimental hutch LCLS



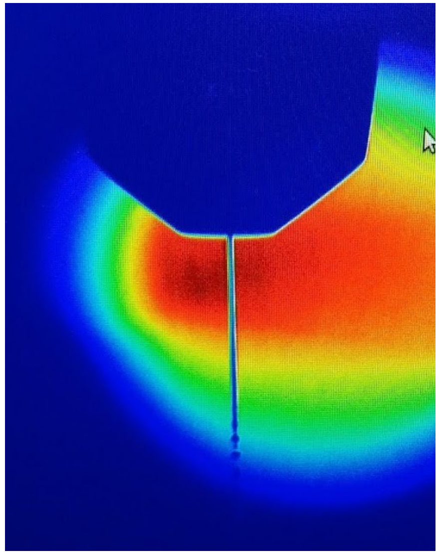
I24 Diamond

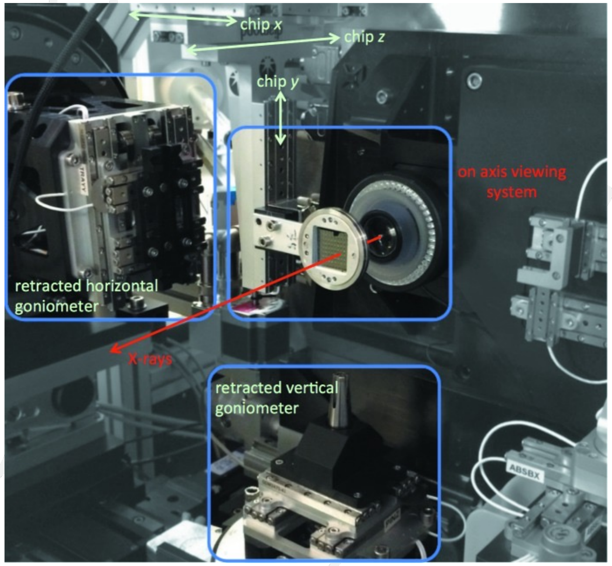
diamond

- ❑ XFEL-Hub introduction
- ❑ X-ray sources: Synchrotron and XFEL
- ❑ **Sample deliveries current and upcoming**
    - High viscosity extruder
    - Fixed target
    - Hybrid method: drop on tape

Sample delivery methods available for SSX

Jet
GDVN, Co-Mesh, mixing injector, **LCP extruder**…

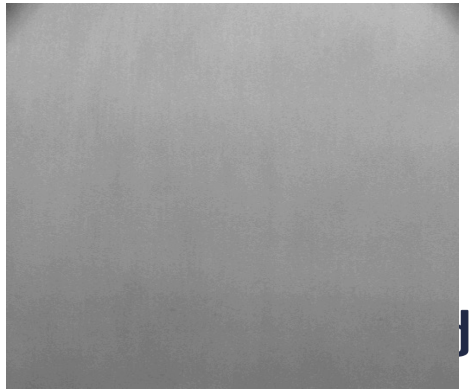




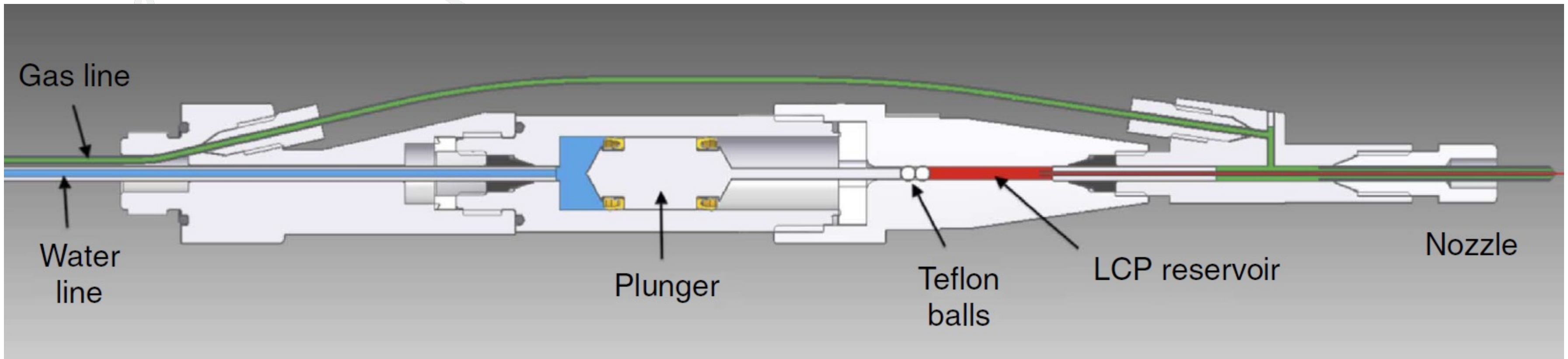
Owen et al., Acta D 2017

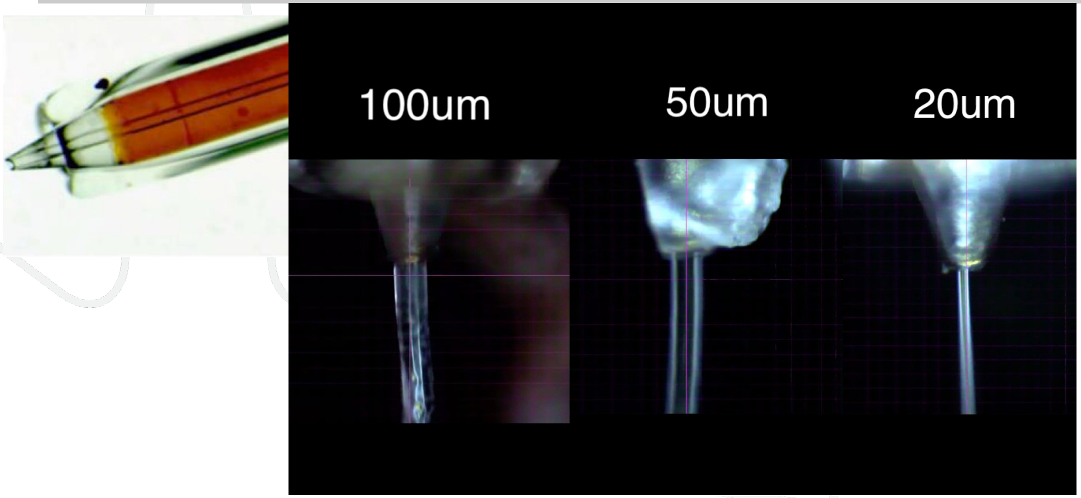
Fixed target
I24's portable station,
Road runner, goniometer…

Hybrid method
Acoustic droplet ejection (ADE) / drop on tape



High Viscosity Extruder





- Low sample consumption <0.3 µl/min
- Crystal size up to 30 – 50 µm
- Can be used at both Synchrotron and XFEL
- Designed for membrane protein in LCP originally
- Install on I24 upon request
- Pump probe experiment

Weierstall et al., Nat Commun (2014)

Zhou et al., Adv Exp Med Biol (2016)

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Fixed target: with a chip

Crystal(s) fixed on sample holder and during data collection the sample holder move to different positions

- <100 µl Crystal solution pipetted over chip made of silicon nitride
- Chip capacity = 25,600
- Funnel-like apertures for holding crystals

Sherrell *et al.,* J Synchrotron Radiat. 2015

- low consumption
- Up to 40 µm crystals
- For XFEL and synchrotron
- Time resolved pump probe and mixing experiment on i24

Drop on Chip (DoC) mixing experiment for time resolved serial crystallography

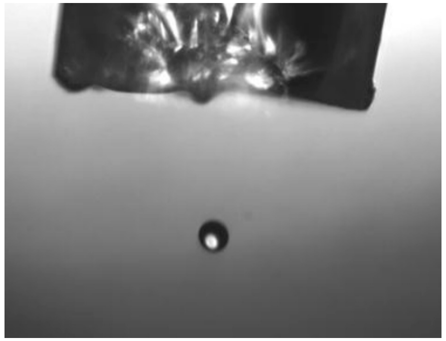
nature methods SUPPLEMENTARY INFORMATION
https://doi.org/10.1038/s41592-019-0553-1
In the format provided by the authors and unedited.

Liquid application method for time-resolved analyses by serial synchrotron crystallography

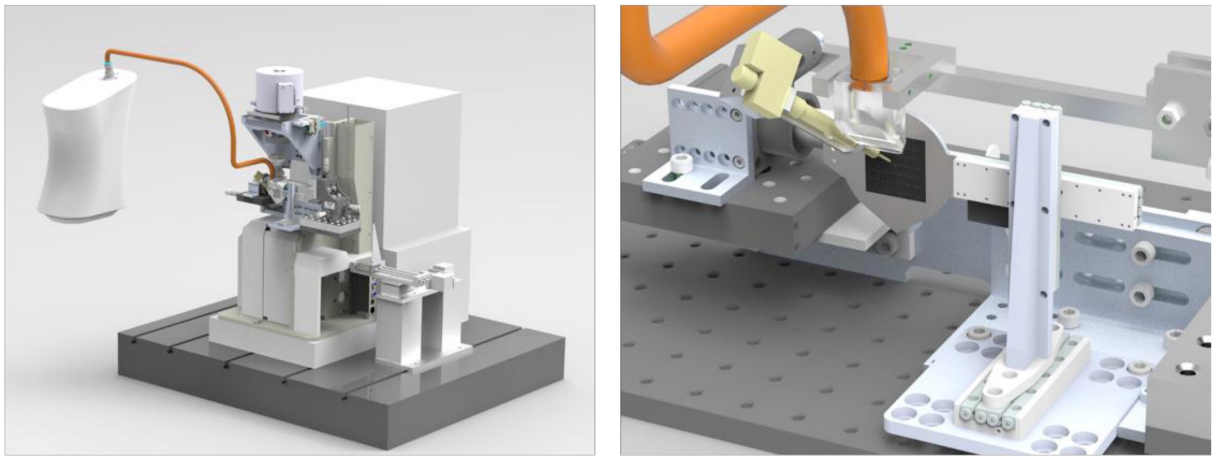
Pedram Mehrabi[1,7], Eike C. Schulz[1,2,7], Michael Agthe[3], Sam Horrell[3], Gleb Bourenkov[4], David von Stetten[4], Jan-Philipp Leimkohl[5], Hendrik Schikora[5], Thomas R. Schneider[4], Arwen R. Pearson[2,3], Friedjof Tellkamp[5] and R. J. Dwayne Miller[1,2,6*]

[1]Max-Planck-Institute for Structure and Dynamics of Matter, Department for Atomically Resolved Dynamics, Hamburg, Germany. [2]Centre for Ultrafast Imaging, Universität Hamburg, Hamburg, Germany. [3]Institute for Nanostructure and Solid State Physics, Universität Hamburg, Hamburg, Germany. [4]European Molecular Biology Laboratory, Hamburg Unit c/o Deutsches Elektronen Synchrotron, Hamburg, Germany. [5]Max-Planck-Institute for Structure and Dynamics of Matter, Scientific Support Unit Machine Physics, Hamburg, Germany. [6]University of Toronto, Departments of Chemistry and Physics, Toronto, Ontario, Canada. [7]These authors contributed equally: Pedram Mehrabi, Eike C. Schulz. *e-mail: dwayne.miller@mpsd.mpg.de

Autodrop pipette
70 µm aperture
~ 180-200 pl
Up to 6kHz

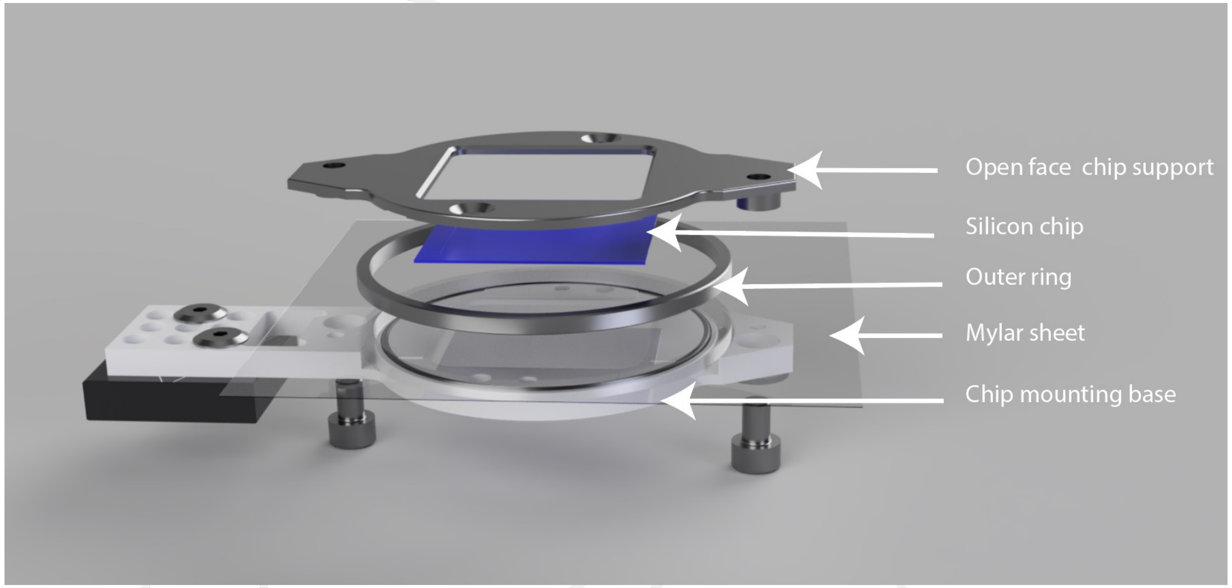


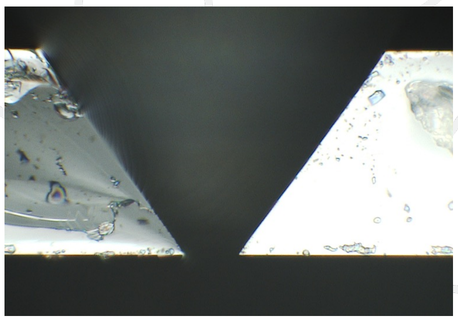
a **b**



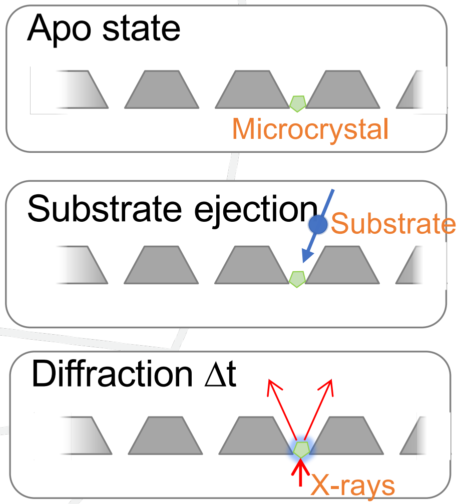
Set up used at the T-REXX beamline (Hamburg)

diamond

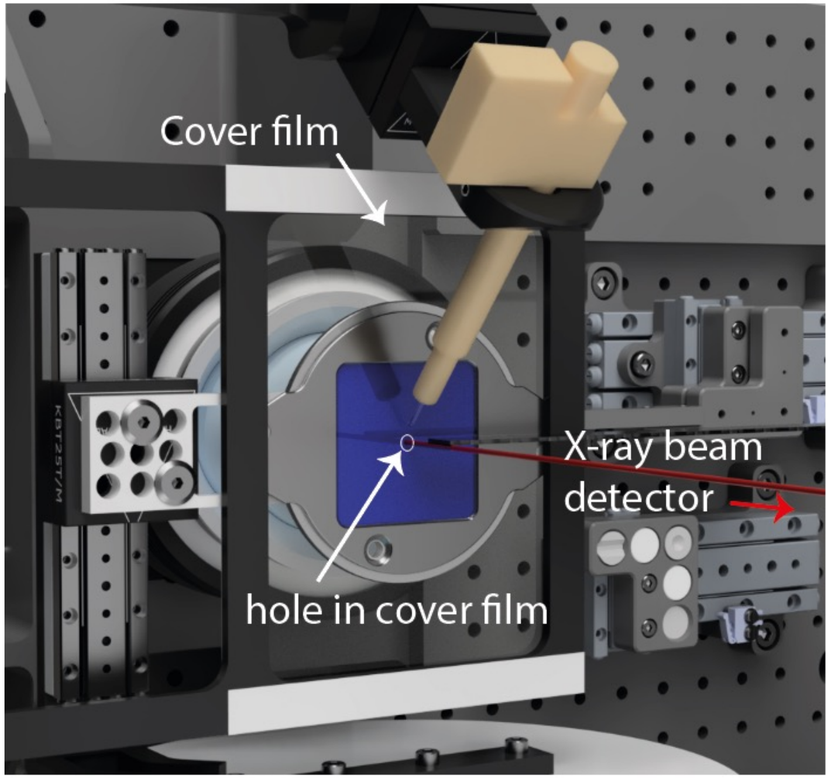




Aperture cross-section
(x2000 magnification)



I24 set-up



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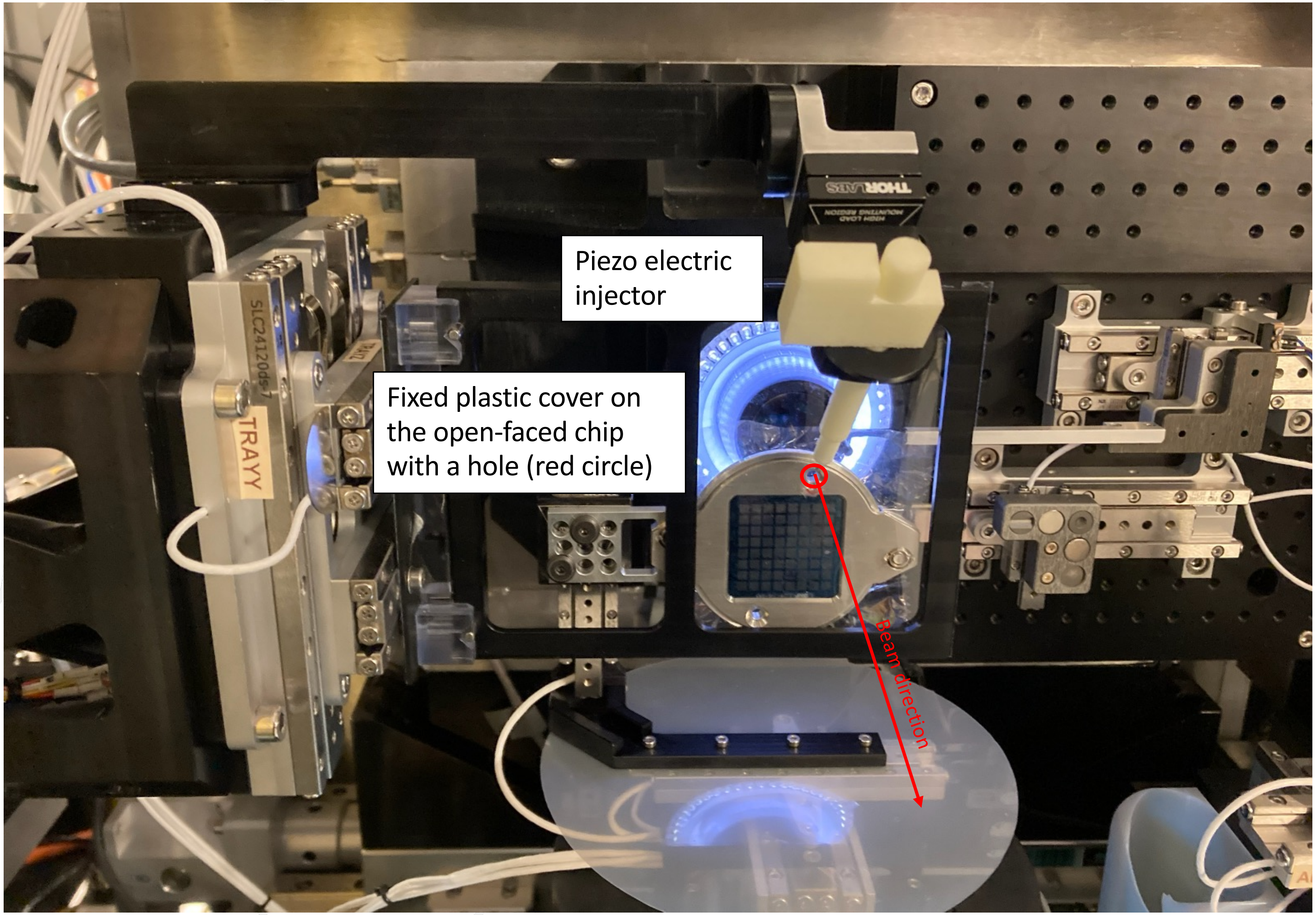
DoC at Diamond i24



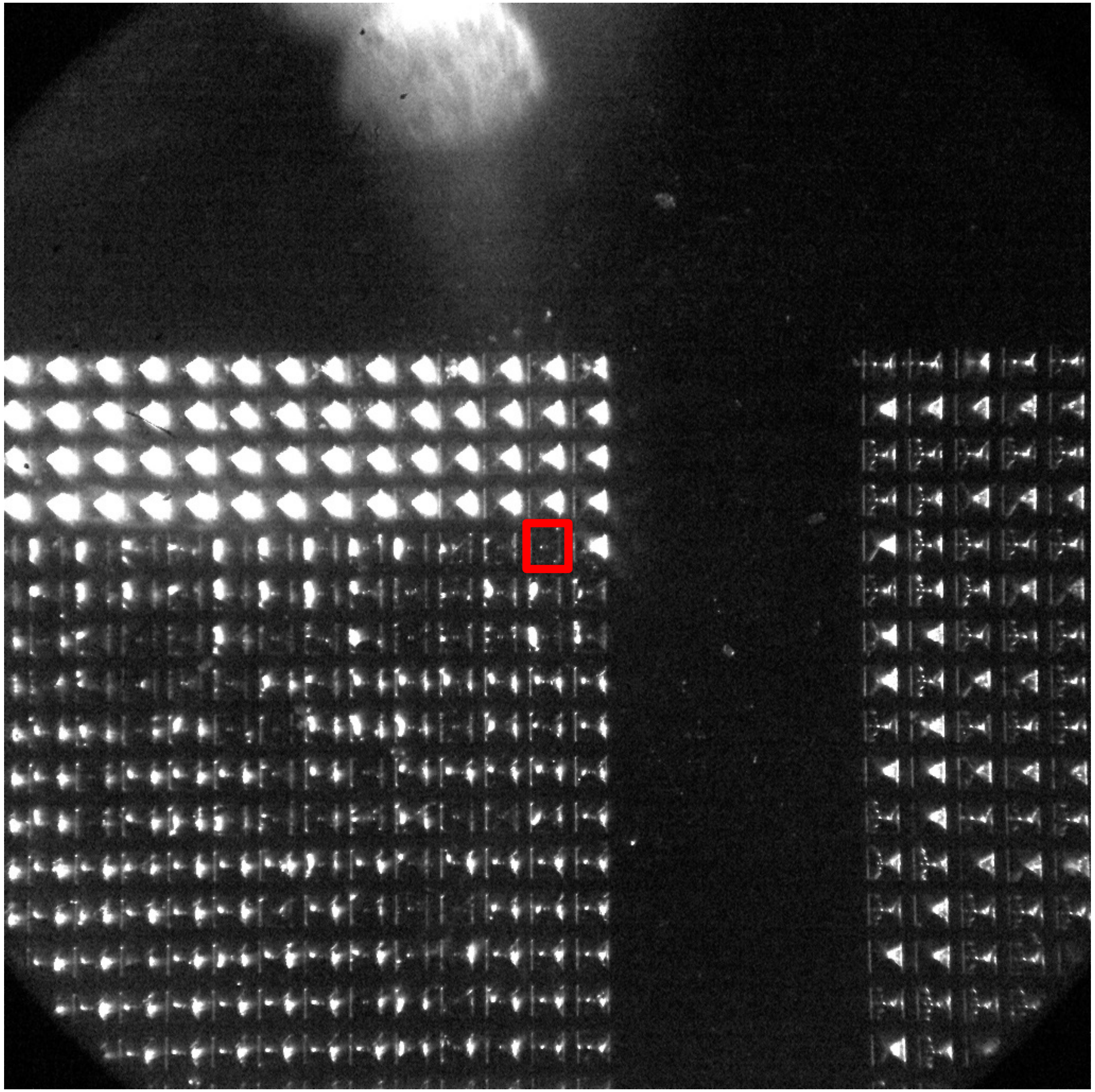
Ejection of substrate on chip

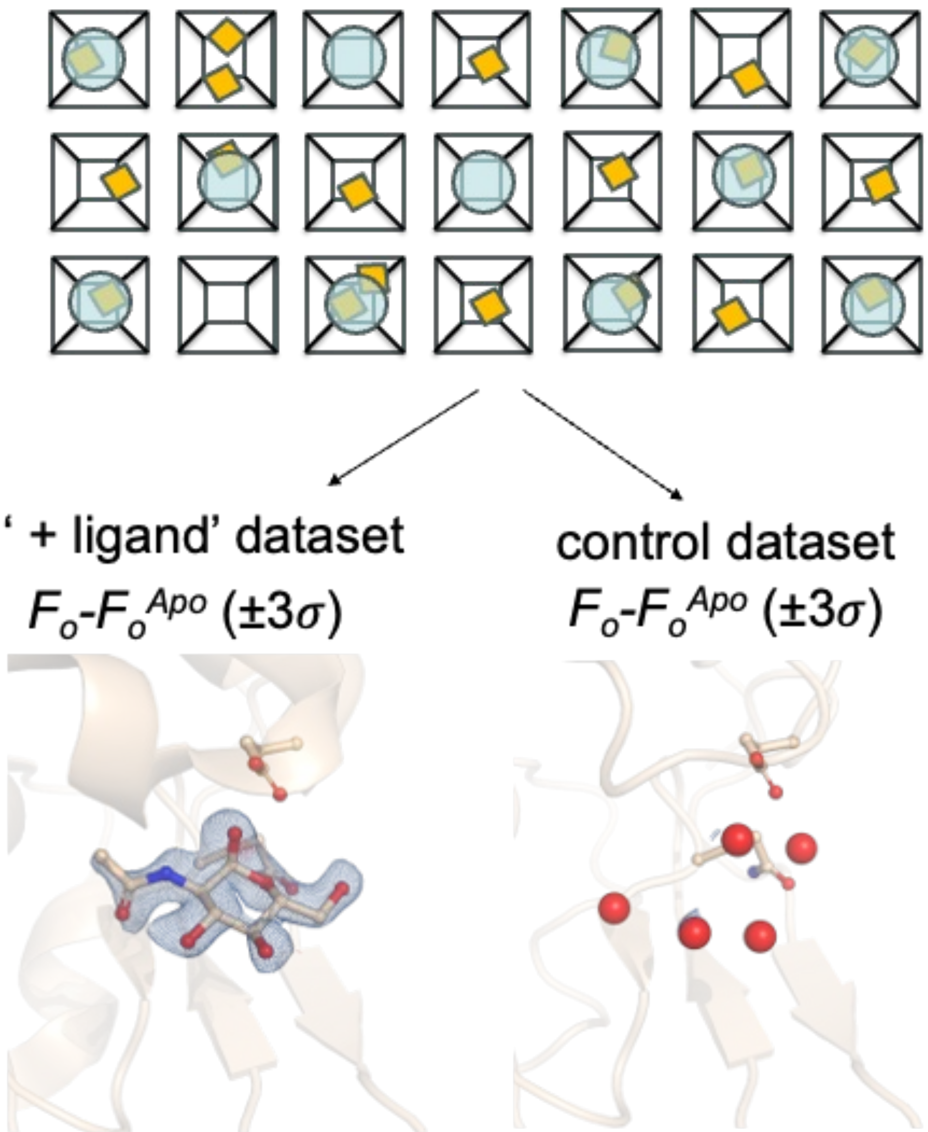
>95 % hit

30 µm ID capillary ~ 50-70 pl drop

10 ms dwell time at each well

About 75 time slower than real time





Lysozyme ± GlcNAc, Δt = 1900 ms

1 chip 2 datasets

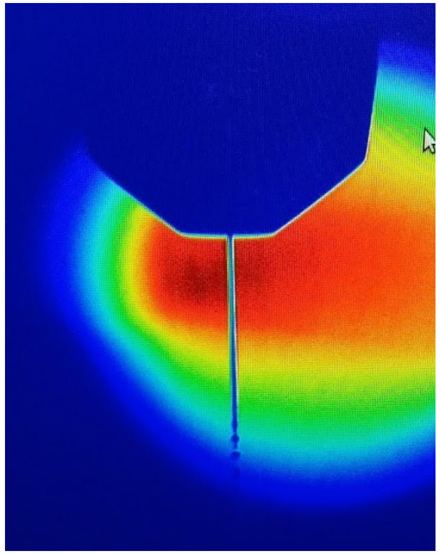
- Interleaved control
- Substrate added

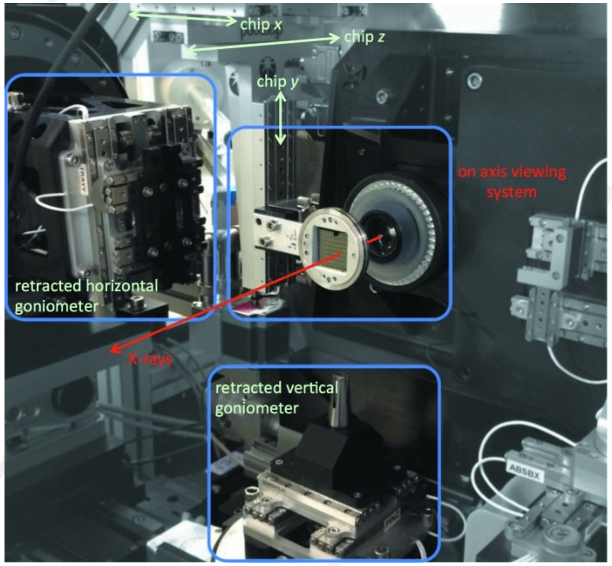
Possible leakage between apertures
=> unreliable Δt

diamond

Sample delivery methods available for SSX

Jet
GDVN, Co-Mesh, mixing injector, LCP extruder…

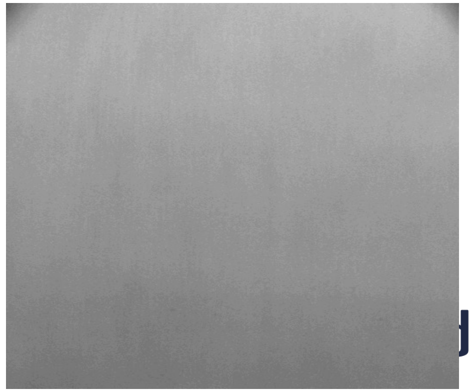




Owen et al., Acta D 2017

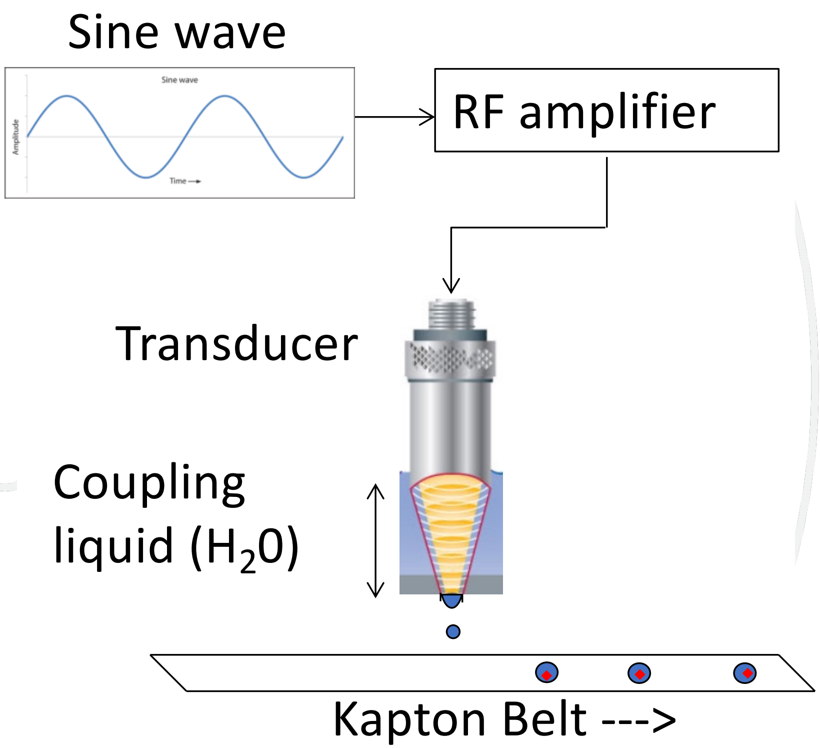
Fixed target
I24's portable station,
Road runner, goniometer…

Hybrid method
Acoustic droplet ejection (ADE)



On demand: drop on tape

hybrid delivery method using both jet (acoustic droplet ejection) and fixed target (tape drive)

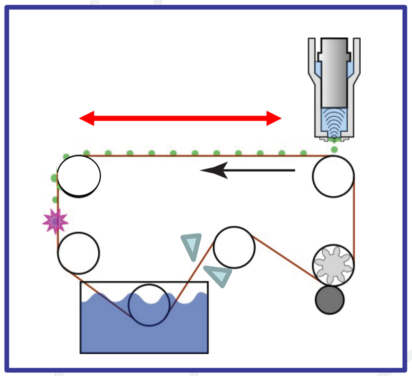


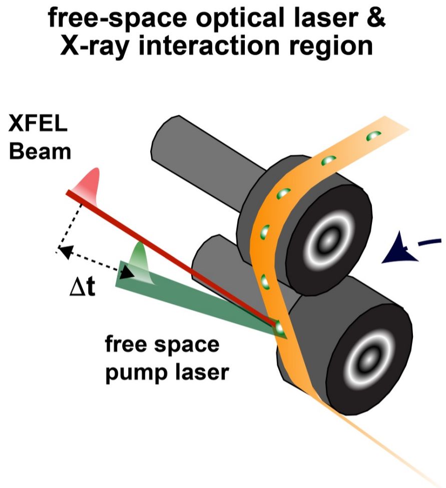
Some Characteristics of Acoustic Droplet Ejection (ADE)

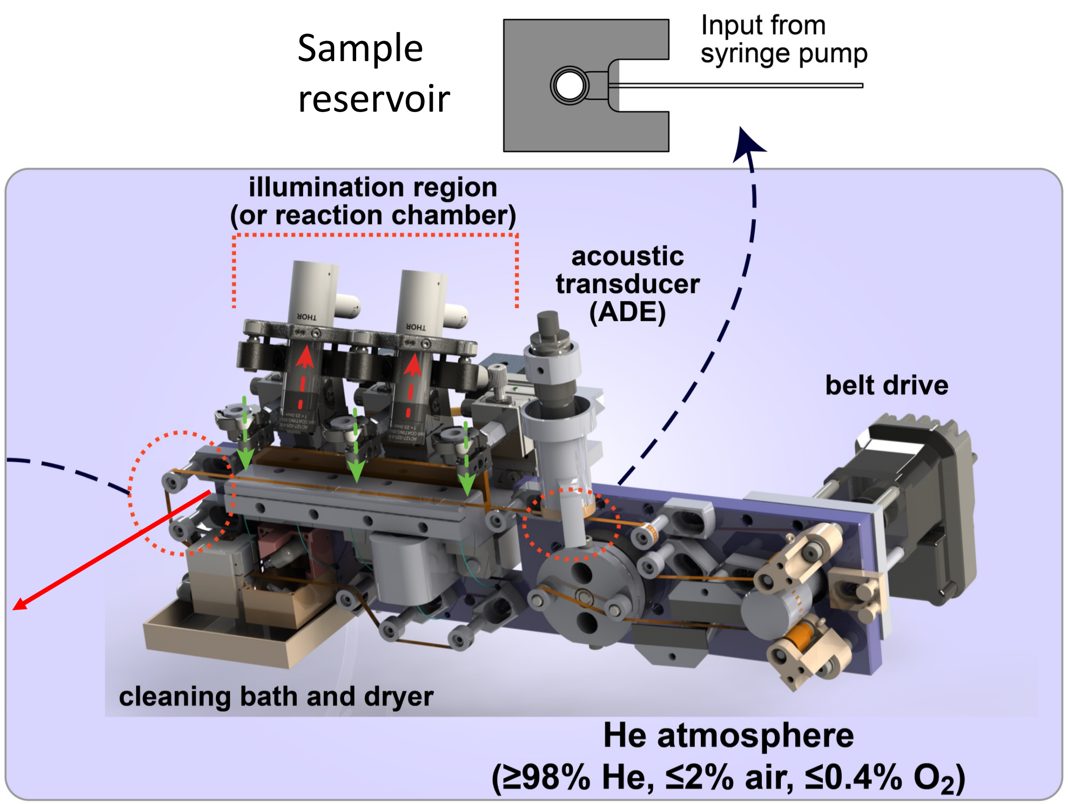
Transducer frequency (MHz)	~ Droplet volume (nL)	~ Droplet diameter (µm)	~ Crystal size within drop (μm^3)
11.5	2.5	170	10 – 100
15	1	125	10 – 75
25	0.1	60	10 – 40

Max ejection frequency ~100 Hz

The ADE-tape drive sample delivery system for tr-SFX ± tr-XES







Fuller and Gul *et al.,* (2017) Nature Methods 14: 443-449
Butryn, Simon, Aller *et al.,* (2021) Nat Commun. 12(1):4461

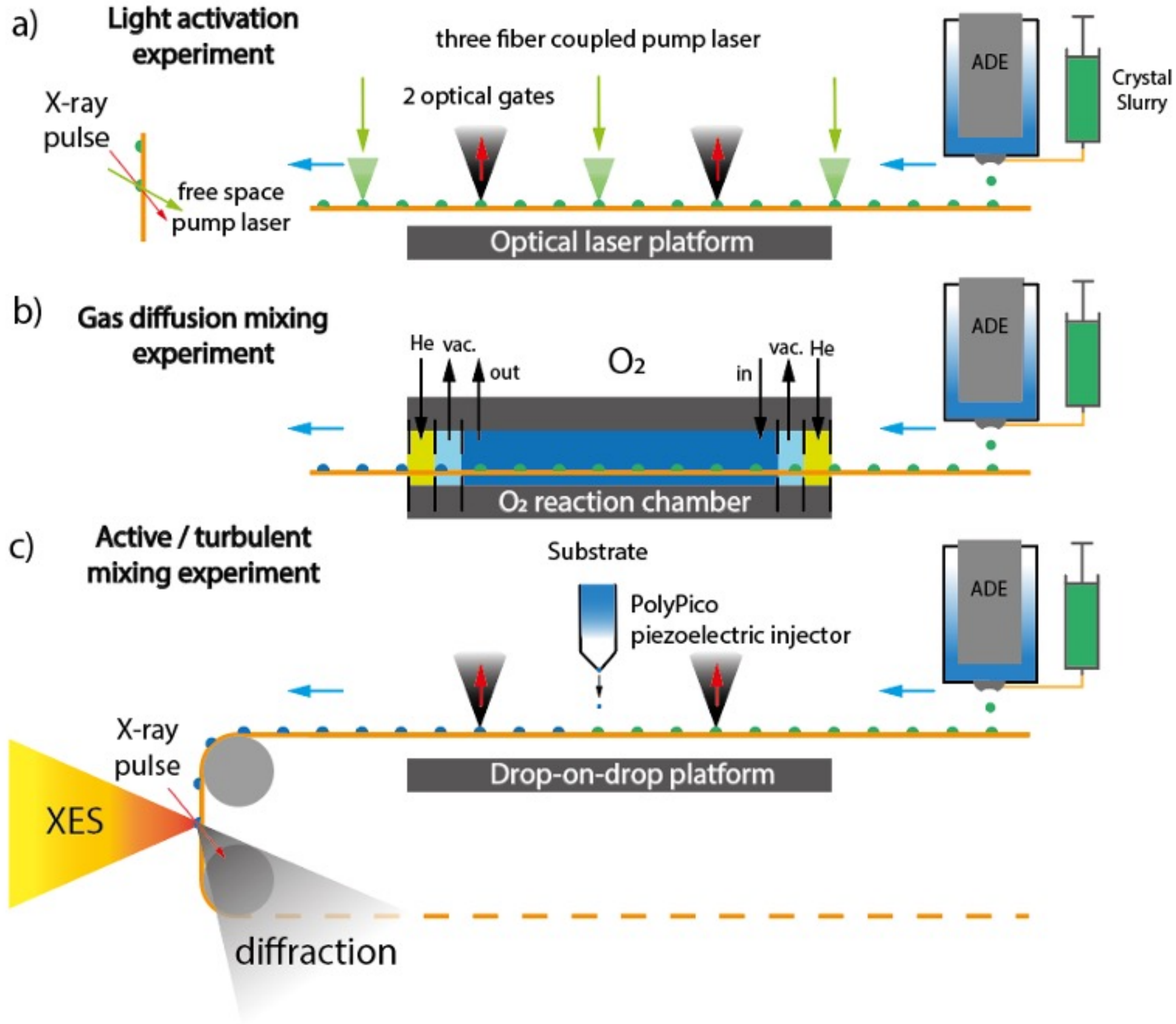
Reaction initiation in crystals:

a) Pump probe: laser illumination

b) Mixing experiment:
O_2 mixing in a reaction chamber

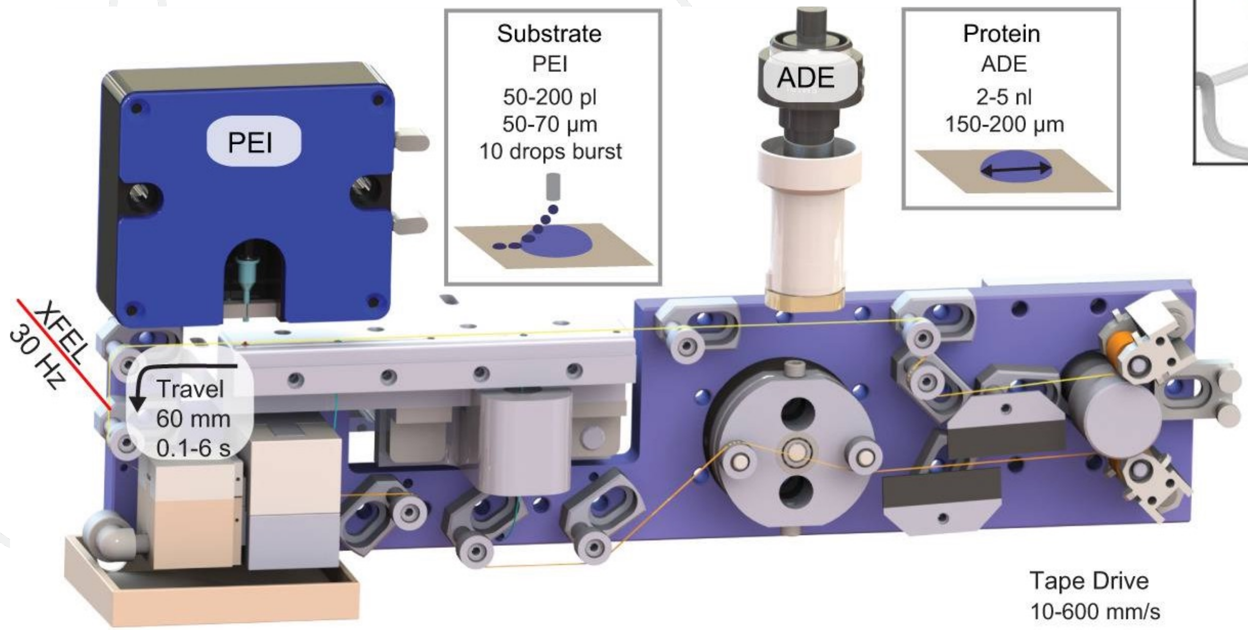
c) Drop on drop induces turbulent mixing

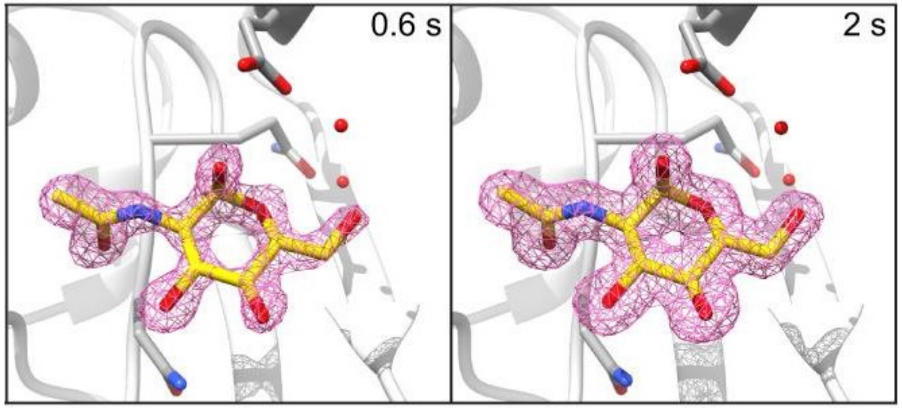
- Various crystal size up to 100 µm
- Wide range of experiments (modularity)
- Low consumption few µl/min
- For XFEL and synchrotron



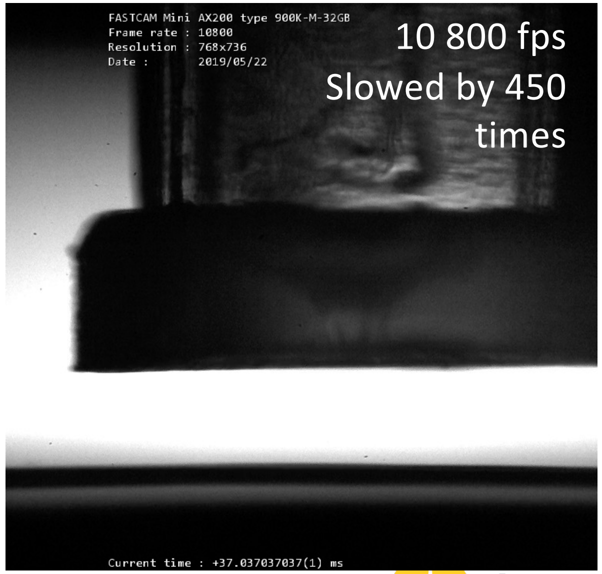
Drop on drop induces turbulent mixing

In 2019 first test of mixing experiment at SACLA





Lysozyme + GlcNAc



Mixing experiment at SACLA and LCLS, Δt = 10 ms – 2s

From Butryn, Simon, Aller *et al.,* Nat Commun. 2021

diamond

Journal of the American Chemical Society > Vol 145/Issue 46 > Article

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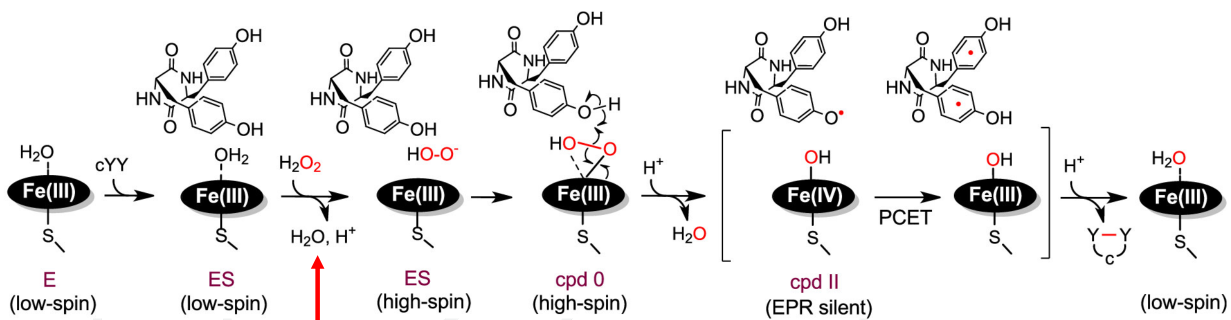
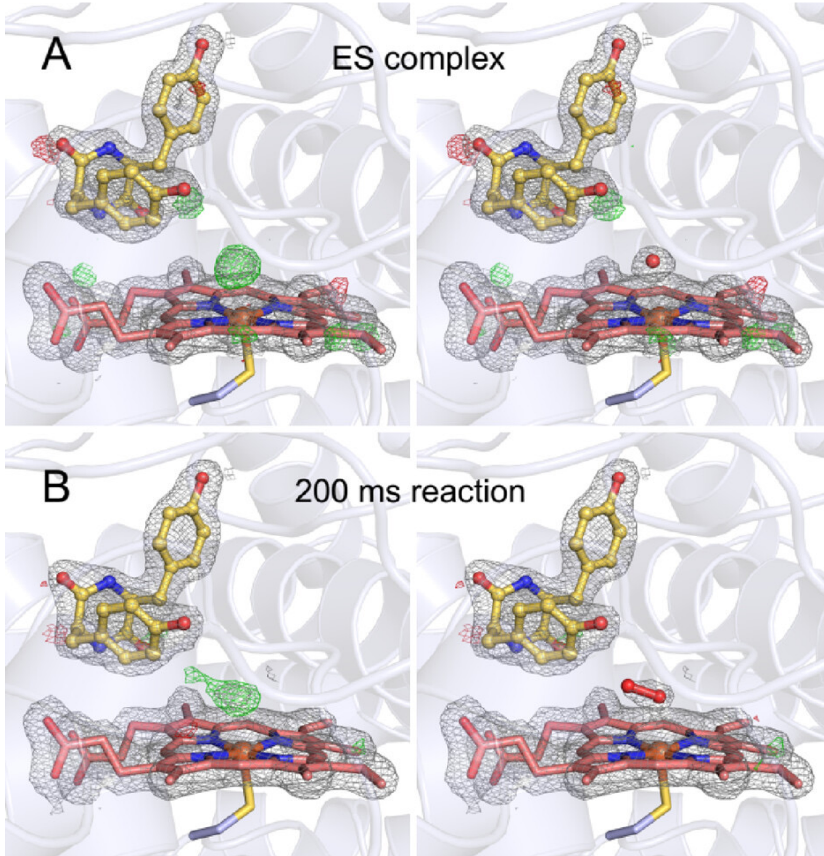
ARTICLE | November 8, 2023

In Situ Structural Observation of a Substrate- and Peroxide-Bound High-Spin Ferric-Hydroperoxo Intermediate in the P450 Enzyme CYP121

Romie C. Nguyen, Ian Davis, Medhanjali Dasgupta, Yifan Wang, Philipp S. Simon, Agata Butryn, Hiroki Makita, Isabel Bogacz, Kednerlin Dornevil, Pierre Aller, Asmit Bhowmick, Ruchira Chatterjee, In-Sik Kim, Tiankun Zhou, Derek Mendez, Daniel W. Paley, Franklin Fuller, Roberto Alonso Mori, Alexander Batyuk, Nicholas K. Sauter, Aaron S. Brewster, Allen M. Orville, Vittal K. Yachandra, Junko Yano, Jan F. Kern*, and Aimin Liu*





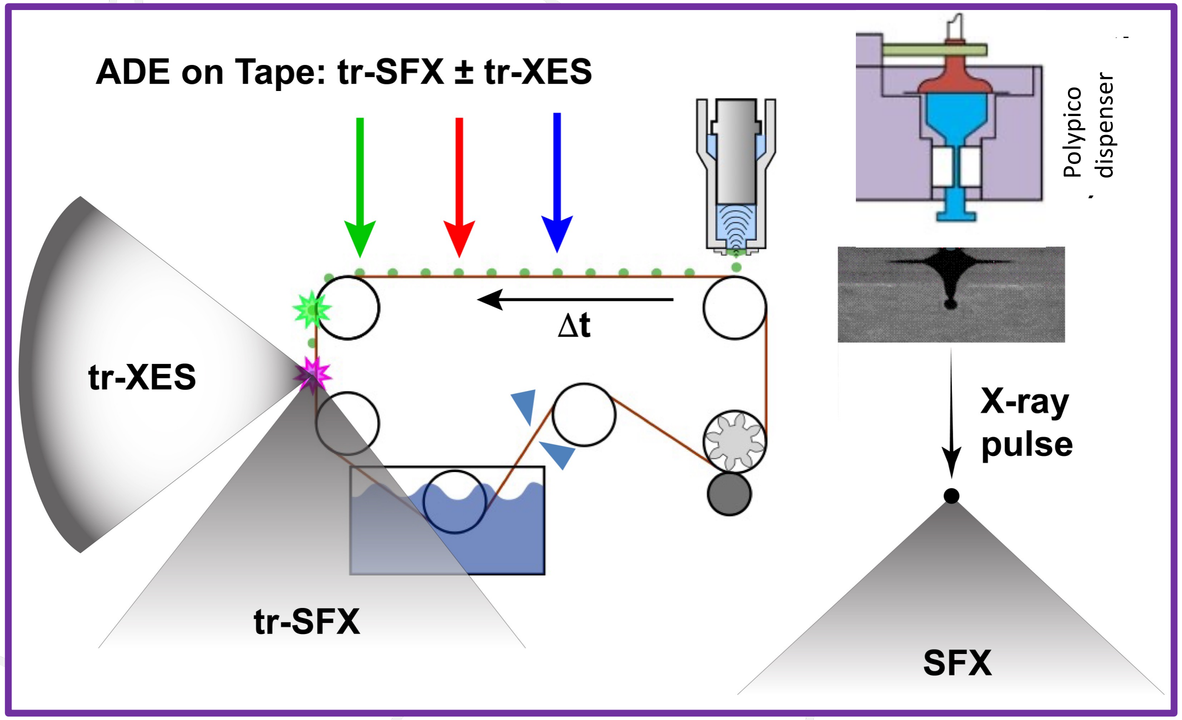
CYP121 + cYY
(no oxidant)

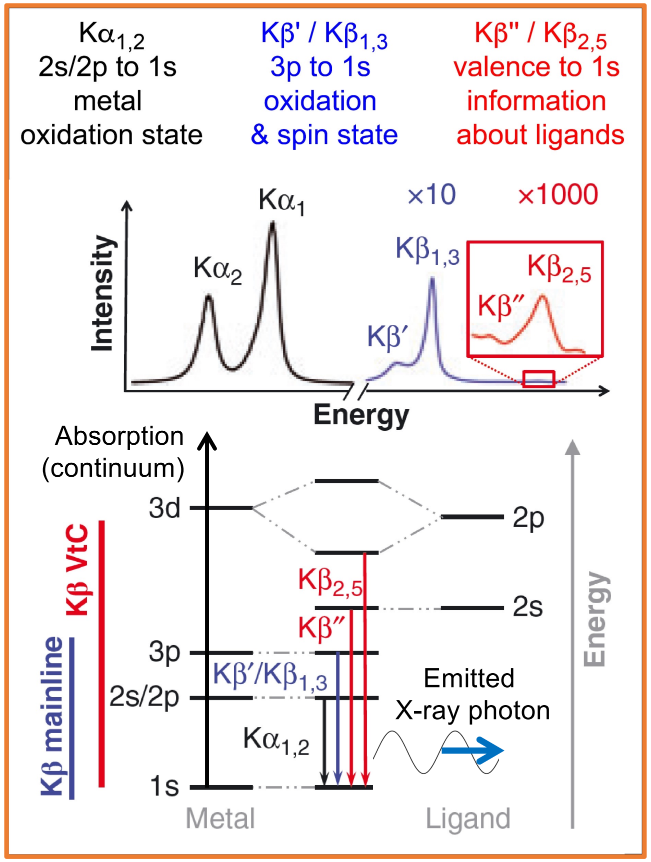
@ 200 ms
hydroperoxo intermediate (cpd 0)

10 drops (250 mM) of PAA at 1.6 kHz
~ 14 mM final (~1.8mM H_2O_2)

cYY: cyclo(l-Tyrosine-l-Tyrosine)

X-ray emission spectroscopy





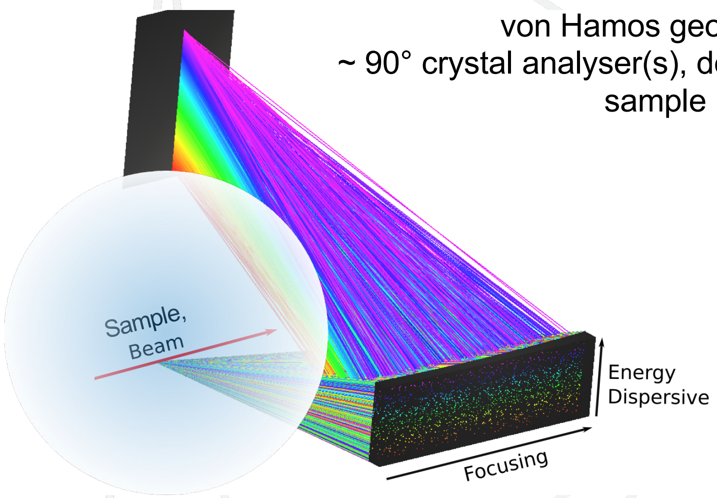
Adapted from
DeBeer & Bergmann (2016)
Encyclopedia of Inorganic and Bioinorganic Chemistry

Applications:

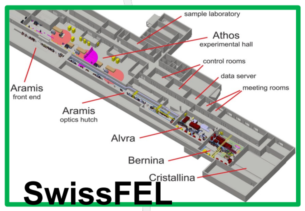
- Probing metal oxidation state for transition metals ($K\alpha_{1,2}$)
- Metal oxidation and spin state ($K\beta_{1,3}$)
- Information about ligands and coordination sphere ($K\beta_{2,5}$)

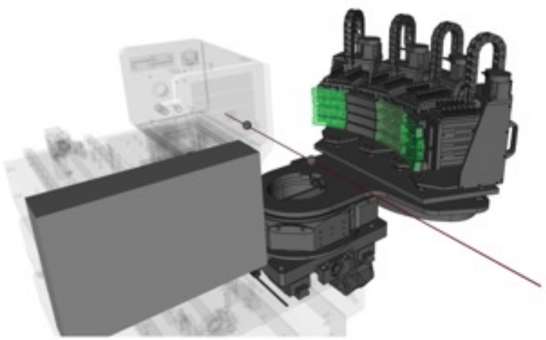
Von Hamos Spectrometer

von Hamos geometry:
~ 90° crystal analyser(s), detector in-plane w/ sample



Cylindrical crystals
→ High energy resolution
→ Metal of interest selected via X-ray Bragg reflection





16-crystal XES analyzer array

Multiple crystals → samples larger spherical solid angle, better signal to noise ratio and higher sensitivity

Crystal array → experimental flexibility

- ✓4x1 array → $K\alpha_{1,2}$
- ✓3x4 or 4x4 array
  - → $Kb_{1,3}$ (&/or $Kb_{2,5}$)

Current scope:

Element	Z	Name	$K\alpha_{1,2}$	$K\beta_{1,3}$	$K\beta_{2,5}$
Fe	26	iron	6405, 6392 eV	7059, 7059 eV	7110 eV
Cu	29	copper	8046, 8026 eV	8903, 8901 eV	8974 eV
Ni	28	nickel	7480, 7463 eV	8266, 8265 eV	8329 eV

Future scope:

Element	Z	Name	$K\alpha_{1,2}$	$K\beta_{1,3}$	$K\beta_{2,5}$
Mn	25	manganese	5900, 5889 eV	6491 eV	6537 eV
Co	27	cobalt	6930, 6915 eV	7649, 7650 eV	7706 eV
Mo	42	molybdenum	17480, 17375 eV	19606, 19588 eV	19962, 19768 eV

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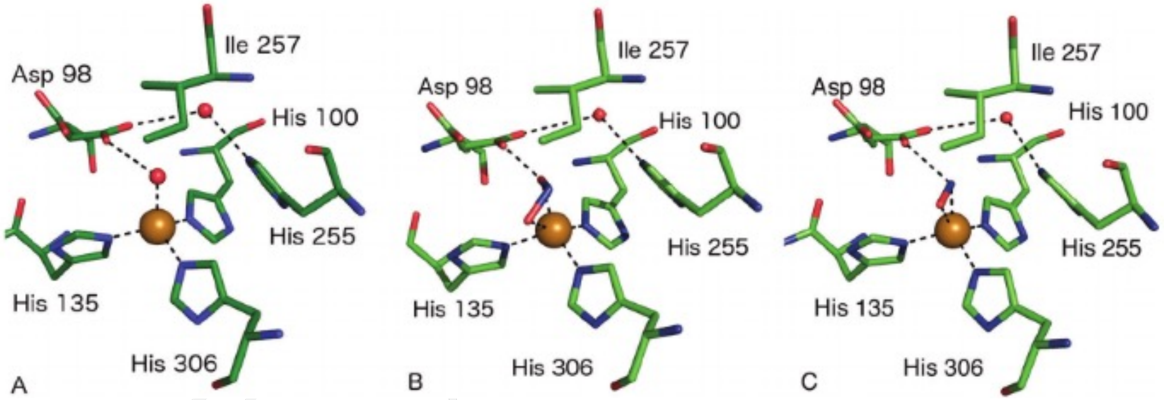
XES + XRD data collected from AcNIR crystals to detect oxidation state of Cu

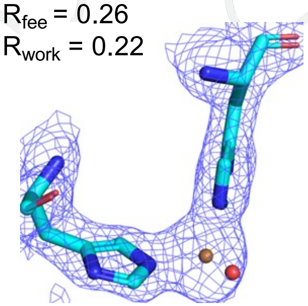
Copper nitrite reductases are key enzymes in the nitrogen cycle.



The denitrification pathway in these organisms couples ATP synthesis with the reduction of nitrite (NO_2^-) to dinitrogen (N_2), via nitric oxide (NO) intermediates. This pathway is critical in the return of fixed nitrogen to the atmosphere and in controlling the level of biologically available nitrogen in the soil.

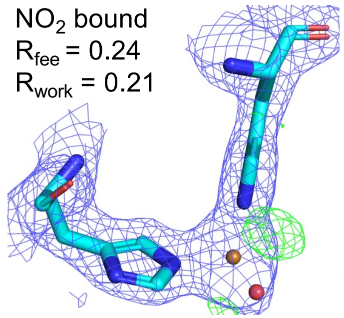
$$NO_2^- + e^- + 2H^+ \rightarrow NO + H2O$$

Apo-state
$R_{fee} = 0.26$
$R_{work} = 0.22$



Dataset name	xia2-dials Integrated	xia2-dials-ransac Integrated	xia2-dials Merged	xia2-dials-ransac Merged	xia2-dials CC1/2 = 0.3	xia2-dials-ransac CC1/2 = 0.3
Apo-state 0.58 Mgy (reduced)	17755	20947	17748	20893	1.9	1.9
NO_2 bound 0.58 Mgy (reduced)	10920	14028	10893	13873	1.94	1.92

NO_2 bound
$R_{fee} = 0.24$
$R_{work} = 0.21$



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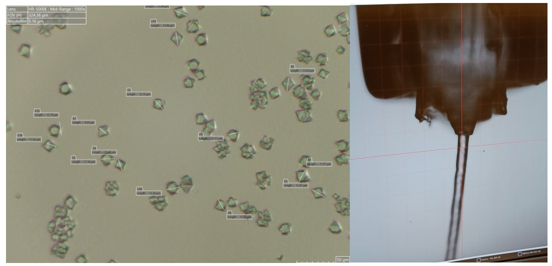
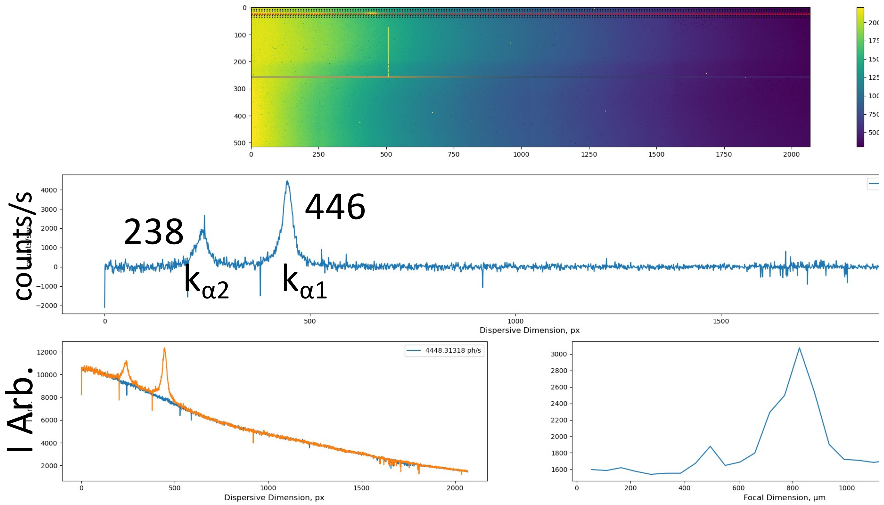
Recent Structural Insights into the Function of Copper Nitrite Reductases (2017)

Acnir + LCP using HVE injector



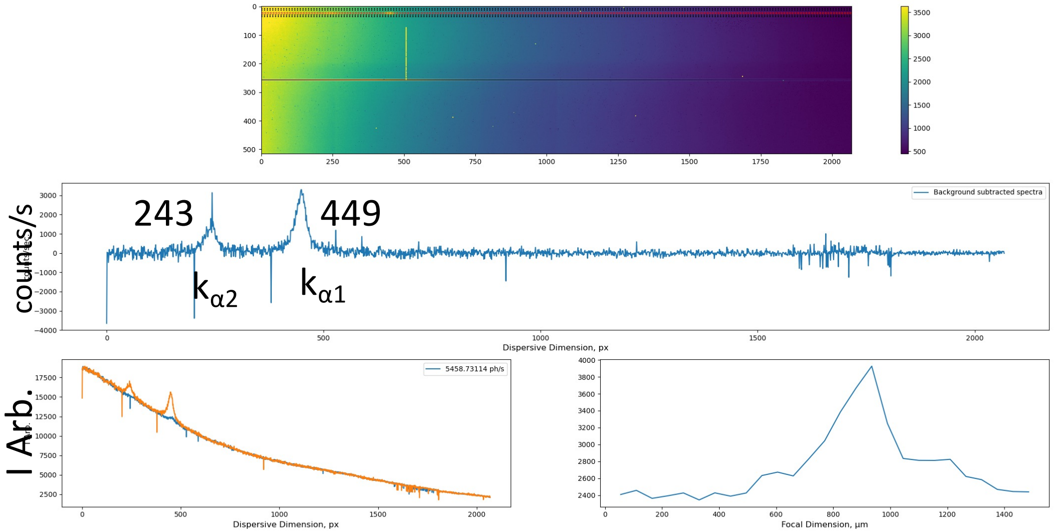
average crystal size 12 µm

Dataset 412-415

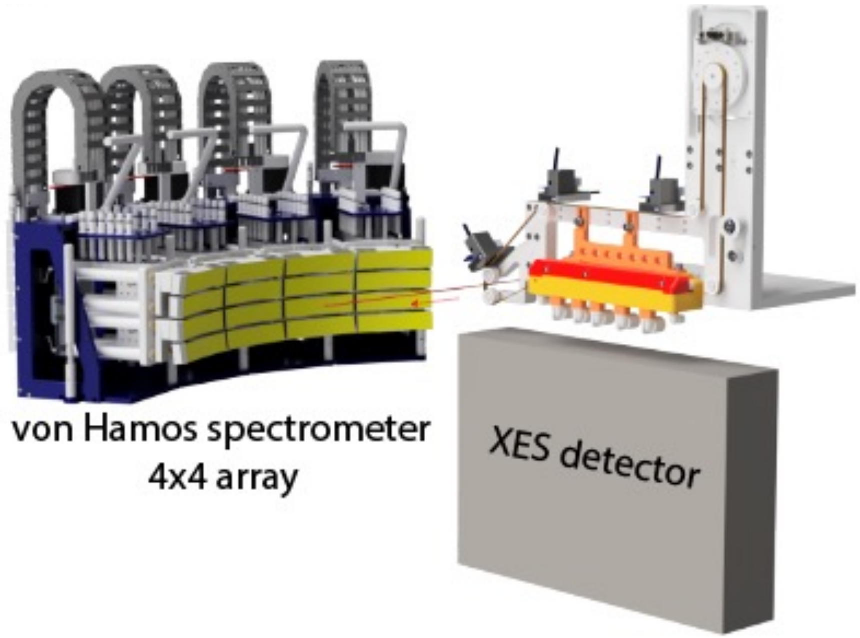


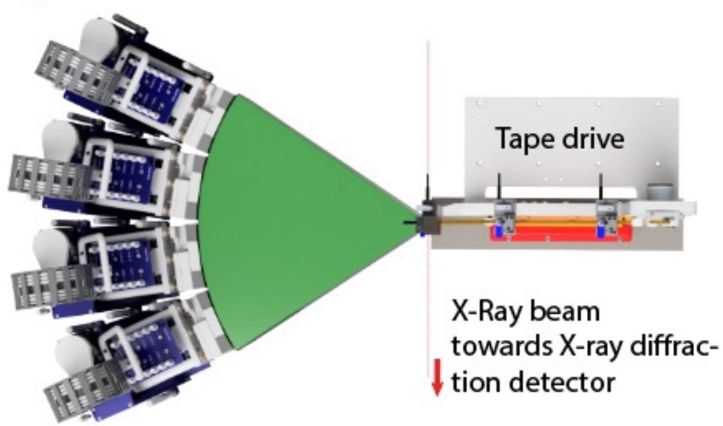
Acnir (Cu I)

Dataset 420-424



Acnir (Cu II)





Kamps JJAG, Bosman R, Orville AM, Aller P. Methods Enzymol. 2024; 709

In conclusion:

$\Rightarrow$ How to choose your sample delivery?

Do I need an XFEL or a synchrotron source?
What do I want to achieve?
How much sample can I produce?
What is the crystal size and density?

If you want to try SSX, please contact us! We can offer beamtime at Diamond through our BAG!

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