BBSRC, MRC, Wellcome (Naismith et al) 2013 – 2019
Funded to join the SFX Consortium at Eu.XFEL and create the Hub

Hub hosted at Diamond from 2013, since 2019 funded by Diamond Light Source and UKRI / STFC

XFEL Hub diamond

Hub led by A. M. Orville since Oct 2015
Currently: Wellcome Investigator, RS Wolfson Fellowship, & UKRI / BBSRC

AMO group	XFEL Hub
Rob Bosman, PhD	Allen M. Orville, PhD
Jos Kamps, PhD	Pierre Aller, PhD
Tiankun Zhou, PhD	Anastasya Shilova, PhD

Anna Bailey, (Jack Stubbs), Emily Freeman, (Johan Glerup), Harold Cannon, Toluwalase Agoro, *etc.*

Research & Development
- XFELs, Diamond, eBIC, RFI, RCaH
- Time-resolved serial MX, SFX & ED
- **A**nti**M**icrobial **R**esistance, especially β-lactam biosynthesis & degradation
- Reactivity in microcrystals
- X-ray emission spectroscopy (XES)
- Microfluidics for microcrystals
- On-demand droplet injectors

Service & Training
- Travel assistance UK scientists
- *Dynamic Structural Biology at XFELs & Diamond* BAG
- Serial MX processing pipelines

Research & Development
- Cryo-EM grid prep methods
- Serial MX at VMXi ± tr-XES
- Data processing pipeline(s)
- Diamond/CLF PORTO project
- Micro-spectroscopy ± MX
- Novel sample delivery methods

1

XFEL Hub supports UK scientist visits to XFELs

Year	UK scientists on expt.	UK scientists XFEL site visits	XFEL Hub scientists site visit
2015*	3	3	3
2016	25	21	19
2017	70	49	22
2018	95	59	34
Total 2015 - 2022	**386**	**206**	**100**

LCLS, SACLA, PAL-XFEL, SwissFEL, & European XFEL

UK grants to the XFEL Hub and/or collaborators

wellcome
THE ROYAL SOCIETY
MRC Medical Research Council
UKRI Science and Technology Facilities Council
UKRI Biotechnology and Biological Sciences Research Council
UKRI Engineering and Physical Sciences Research Council

Some key publications with the XFEL Hub

nature
Science
nature methods
nature communications
Science Advances
PNAS
Structure
JACS
ACS Catalysis
Angewandte International Edition Chemie
The Journal of Physical Chemistry B

Young et al. (**2016**) *Nature 540*, 453-457
Kern et al. (**2018**) *Nature 563*, 421-425
Lebrette et al. (**2023**) *Science* 382, 109
Fuller et al. (**2017**) *Nature Methods 14*, 443-449
Butryn et al. (**2021**) *Nature Commun 12*, 4461
Wiedorn et al. (**2018**) *Nature Commun 9*, 4025
Rabe et al. (**2021**) *Science Advances 7*, eabh0250
Burgie et al. (**2020**) *Proc Natl Acad Sci USA 117*, 300-307
Ibrahim et al. (**2020**) *Proc Natl Acad Sci USA 117*, 12624-12635
Roessler et al. (**2016**) *Structure 24*, 631-640
Srinivas et al. (**2020**) *J Am Chem Soc 142*, 14249-14266
Lucic et al. (**2022**) *ACS Catal* 12, 13349-13359
Lucic et al. (**2020**) *Angew Chem Int Ed Engl* 59, 21656-21662
Baxter et al. (**2022**) *J Phys Chem B 126*, 9288-9296

2

Most structural biology data are measured from a crystalline sample held at 100 K (often at synchrotrons, including Diamond Light Source)

209,159 atomic models released by the PDB (03 Sep 2023)

Atomic Models in PDB (03 Sep 2023)	
X-ray crystallography	177,905
Cryo-EM (soln.)	16,845
NMR spectroscopy (soln.)	14,070
XFEL data	777
Micro-ED (cryo.)	230
Neutron diffraction	219
"Pharma vaults"	big #

diamond

3

100 K is

… a bit warmer than the surface of ***Pluto***
33 – 55 K; average 44 K

… similar to
The dark side of the **Moon**
~ 90 K

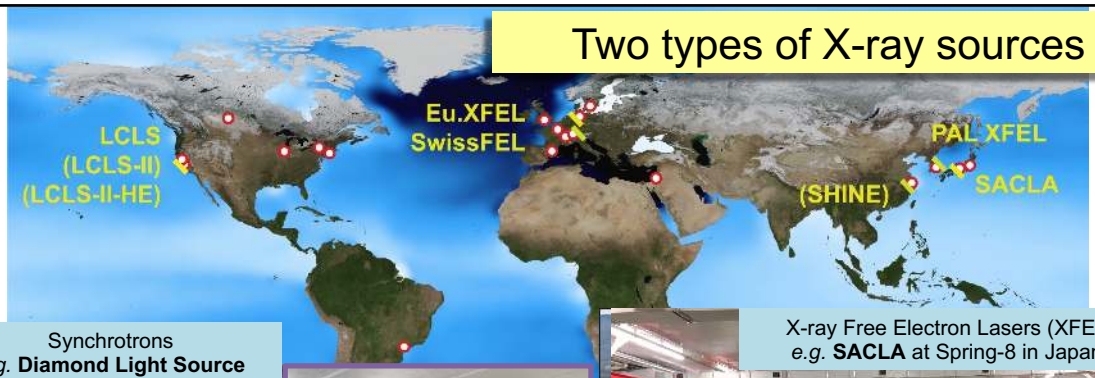
… the temperature of most crystal structures in the PDB

… and so, ***remaining frontier challenges*** in structural biology are to determine
time-resolved, atomic resolution structures
directly from systems engaged in catalysis,
perhaps even in native context / environment,
and certainly, at physiological temperature and pressure
…… ***on earth***

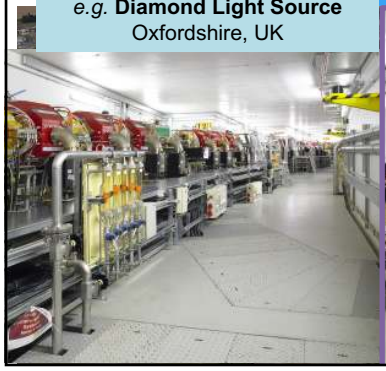
24 Dec 1968
NASA / Apollo 8
AS08-14-2383

4

Two types of X-ray sources



Synchrotrons
e.g. **Diamond Light Source**
Oxfordshire, UK

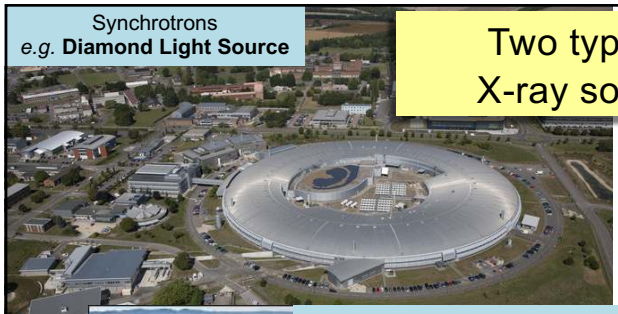


X-ray Free Electron Lasers (XFEL)
e.g. **SACLA** at Spring-8 in Japan)

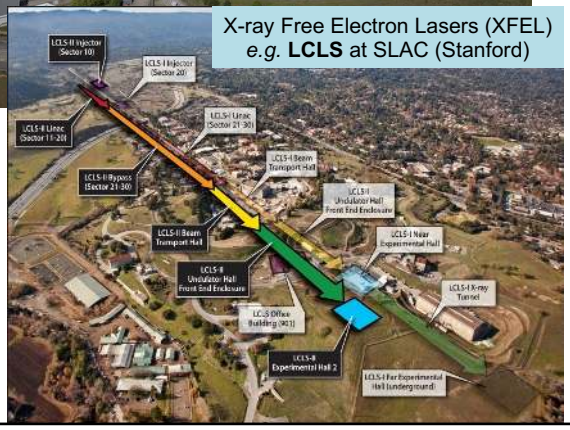
5

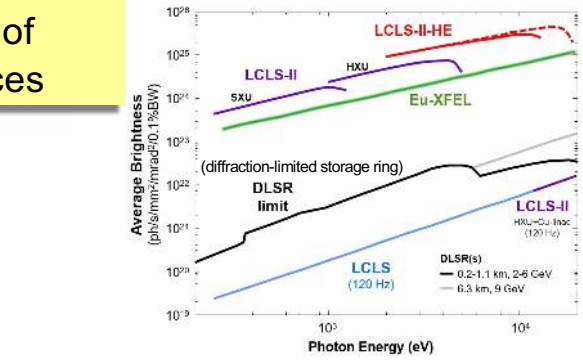
Two types of X-ray sources

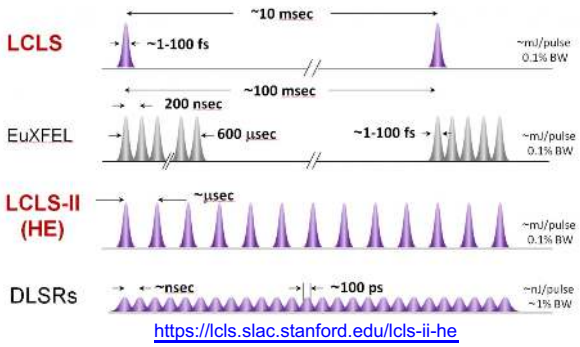
Synchrotrons
e.g. **Diamond Light Source**



X-ray Free Electron Lasers (XFEL)
e.g. **LCLS** at SLAC (Stanford)





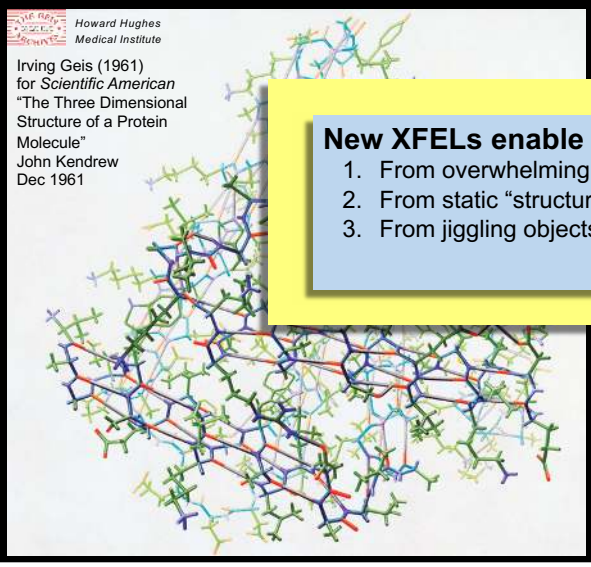


https://lcls.slac.stanford.edu/lcls-ii-he

6

"… all things are made of atoms; everything that living things do, can be understood in terms of the jigglings and wigglings of atoms."

Richard P. Feynman
Lectures on Physics Vol 1, Chapter 3, © 1963

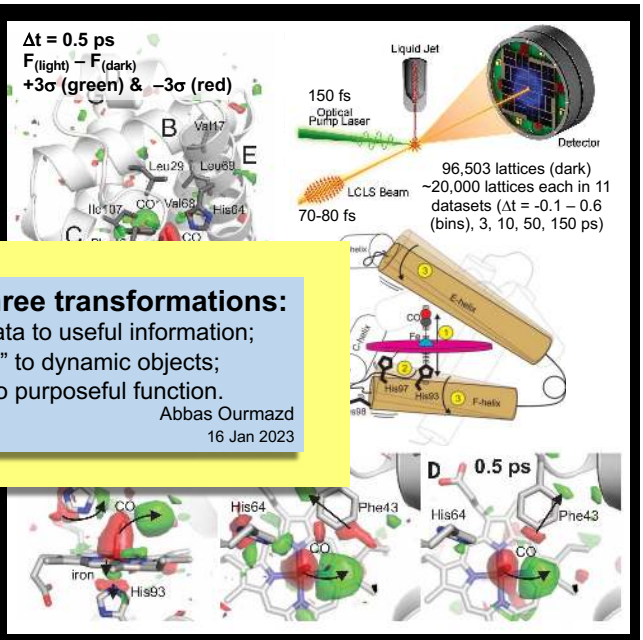


Irving Geis (1961)
for *Scientific American*
"The Three Dimensional Structure of a Protein Molecule"
John Kendrew
Dec 1961

New XFELs enable three transformations:

1. From overwhelming data to useful information;
2. From static "structures" to dynamic objects;
3. From jiggling objects to purposeful function.

Abbas Ourmazd
16 Jan 2023



Mb-CO photodissociation: **time-resolved SFX**
Barends et al (2015) *Science 350*, 445-450

7

Current 3rd generation synchrotrons and XFELs

Parameter / XFEL	LCLS (USA)	SACLA (Japan)	PAL XFEL (Korea)	SwissFEL (Switzerland)	Eu.XFEL (Germany)	LCLS-II-HE (USA)	SHINE (China)
first beam	2009	2011	2017	2017	2017	~2025	~2026
photons / pulse	~ 10^{12}	2 x 10^{11}	>1 x 10^{11}	7 x 10^{11}	~ 10^{12}	≥ 10^{12}	≥ 10^{12}
max. pulses / s	120	60	60 - 120	100	27,000* (4.5 MHz)*	1,000,000 (1 MHz)	1,000,000 (1 MHz)
Detector(s) (image rate, Hz)	CSPAD / Rayonix (120 / 10 - 30)	MPCCD (60)	MPCCD (60)	Jungfrau (100 +)	AGIPD (3520) Jungfrau* (160)	ePIX (R&D) (10,000)	tbd (R&D) (17,000)
typical X-ray beam size (μm²)	3 x 3	3 x 3	5 x 5	5 x 5	3 x 3	tbd	tbd
SFX stations	3	1	1	1	2	3	tbd

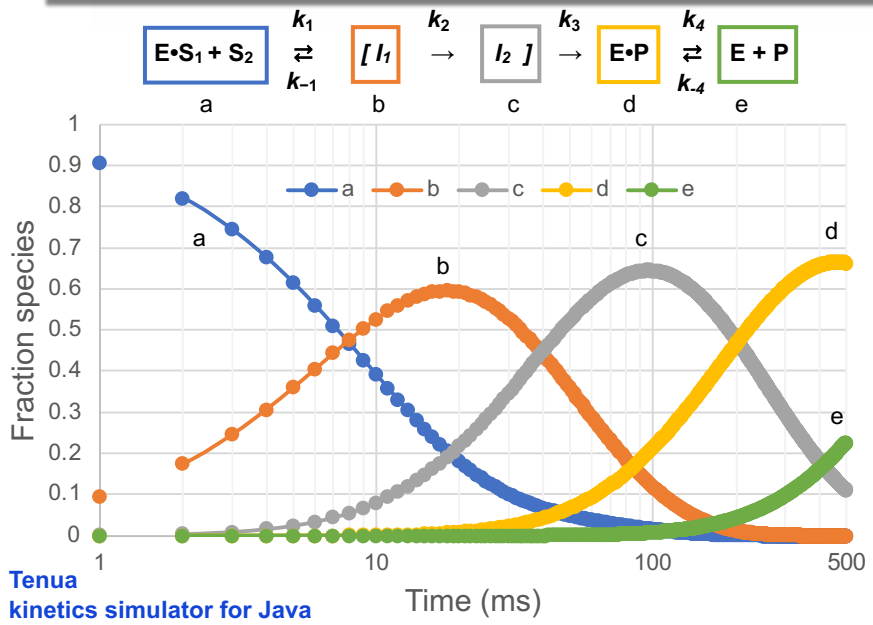
8

Emerging capabilities for tr-SSX at synchrotrons

Beamline	Focus μm²	Flux ×10¹³ ph s⁻¹	Energy keV	Detector(s)	Sample delivery	Other key features
I24 / KMX Diamond, Diamond II	4×4 to 30×30	~0.2 (DCM) **~8 (DMM)** **~100 (D-II)**	7 – 30	CdTe Eiger2 9M, Integrating tbc, Time-resolved px (Tristan 10Mpx)	Cryo pin, fixed target, viscous extruder, drop on demand	XES + optical spec., Dual DCM DMM, PORTO, Chopper shuttering
MicroMAX, Max IV	1×1 to 10×10	0.8 (DCM) **~100+ (DMM)**	5 – 25	CdTe Eiger2 9M, Integrating tbc	Cryo pin Fixed target. Other SSX tbc	Dual DCM DMM, Chopper shuttering
ID29, ESRF-EBS	0.5×0.5 to 5×6	**~100+ (DMM)**	10 – 25 and 35	Jungfrau 4M	Fixed target, tape drive extruder	Chopper shuttering, Flexible sample environment
FMX, NSLS-II	1×1.5 to 10×10	0.4 (12.4 keV)	5 – 30	Eiger2 16M	Cryo pin. Serial (fixed target) in development	Two goniometers
P14-EH2, PETRA-III "T-REXX"	15×10	0.2 (12.7 keV)	12.7	CdTe Eiger 16M (EH1) Eiger 4M (EH2)	Cryo pin (EH1) Fixed targets (EH2)	Parameters are for EH2, **Δ temp**, Laser excitation available

10

Transient kinetics measures individual rate constants



Tenua
kinetics simulator for Java

Stopped-flow
Electronic Absorption

k_1 = 100 mM^{-1} s^{-1}
k_{-1} = 10 s^{-1}
k_2 = 25 s^{-1}
k_3 = 5 s^{-1}
k_4 = 1 s^{-1}
k_{-4} = 0.01 mM^{-1} s^{-1}

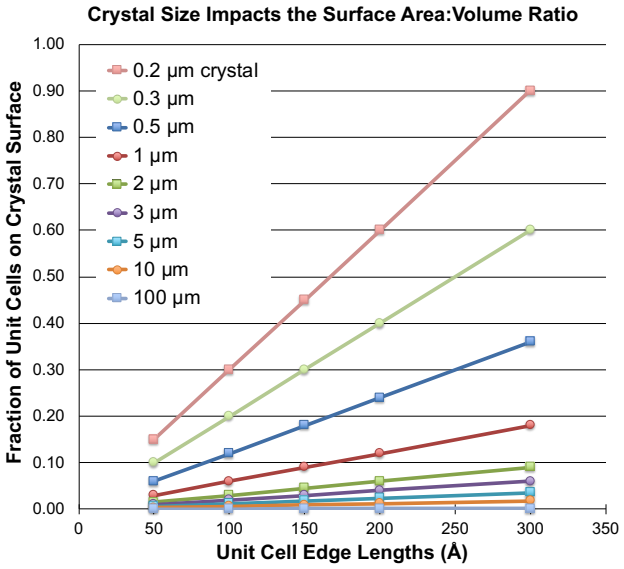
Light → Detector

UV/vis absorption fluorescence, CD

also: Freeze Quench, Chemical Quench

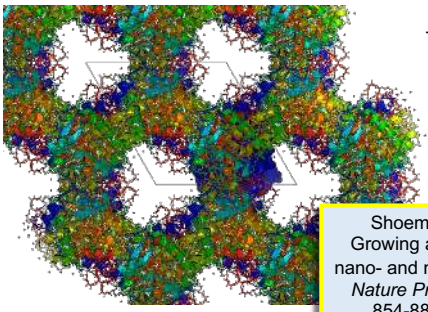
11

The driving hypothesis to ***generalize*** time-resolved serial micro-MX



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

- use **enzyme microcrystals**, ~2x2x2 µm^3 and smaller, homogeneous size distribution
- substrate(s) diffusion is "fast," **E•S complex will form in ~ 100s µs – ms**
- average enzyme reaction in solution is ~ 70 ms; 60% with k_{cat} between 1 – 100 s^{-1}
- Thus, **ligand diffusion is many times faster than typical reaction cycle**



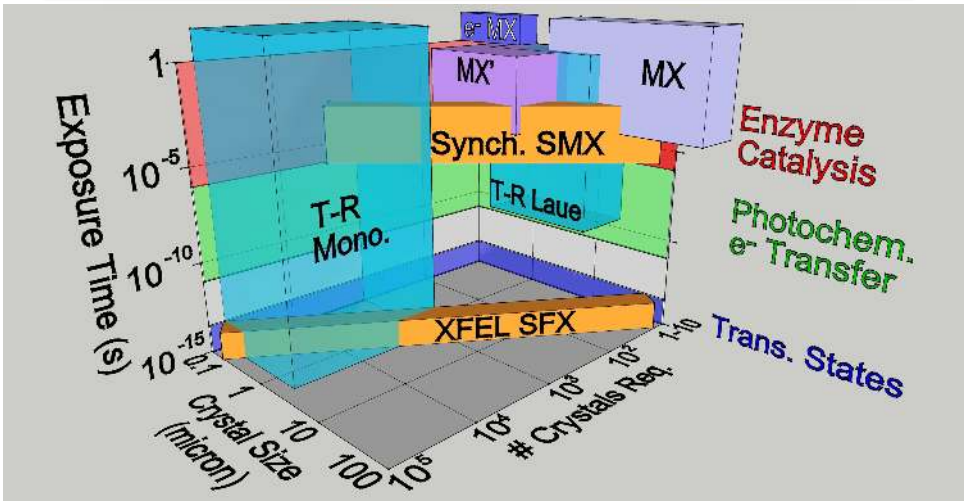
T4 Lysozyme
56% solvent
(PDB 253L)

Shoeman, *et al.* Growing and making nano- and microcrystals. *Nature Protocols* 18, 854-882 (2023)

Davidi D. *et al.* (2018) *Chem Rev* 118, 8786-8797
Schmidt, M. (2017) *Methods Mol Biol 1607*, 273-294

12

Structural biology experimental envelopes



Synchrotron SMX (serial, still (micro)-MX)
XFEL SFX (serial femtosecond MX)
T-R Mono (general, monochromatic, still, time-resolved, micro-MX)

MX (standard, single crystal, rotation MX)
MX' (multicrystal, rotation MX)
e⁻ MX (electron diffraction MX)
T-R Laue (polychromatic, still, time-resolved MX)

18

Concepts of time-resolved *dynamic* structural biology

$$E + S_1 \rightleftarrows E{\bullet}S_1 + S_2 \rightleftarrows E{\bullet}S_1{\bullet}S_2 \rightleftarrows [I_1 \rightarrow I_2 \rightleftarrows I_3 \rightarrow \ldots] \rightarrow E{\bullet}P \rightleftarrows E + P$$

Traditional MX: synchrotrons, macro-crystals, 100 K, resting state **E**, soaked **E•S**$_1$ or **E•P**; soaking or crystallization lacks function and dynamics: >90% structures PDB / year

Cryo-EM: in solution, low Temp or freeze-quench ≈ ms time resolution, complements & benefits from MX, class averages, limited dynamics, no spectroscopic confirmation

Serial MX at XFELs & Diamond (SFX & SMX)
- study entire reaction cycles at room temp & pressure
- XFEL fs pulse ≈ bond vibrations, photo-active reactions
- No radiation-induced damage to reactive intermediates
- DLS / VMXi ≈ μs time resolution with mixing strategies
- μ-crystal slurries ≈ atomic & electronic structural data

Entering an era of *dynamic* structural biology*…* a concept, a set of tools, to collect as much data as possible from every sample and X-ray pulse, enables atomic resolution "stop-motion movies" of macromolecules engaged in catalysis

AIM: Within 5 – 10 years, routine molecular movies via serial MX at all XFELs & synchrotrons

19

Dynamic structural biology → observing structure & function

An ideal experiment…	How & Where ?	What do we need ?
One or more correlated methods tr-SMX/SFX ± tr-XES ± optical ± other X-ray spectroscopies	Integrated instrumentation, Synchrotrons, XFELs, (EM &/or MeV-ED facilities)	Data tracking, analysis, feedback, deep datasets (Δt and ground-state); joint refinement strategies
Under physiological conditions Temp, pressure, pH, salt …	pump-probe, illumination, reagent mixing; equilibration Δt range	Catalytic rates in crystal slurries; efficient sample delivery methods
High spatial resolution *e.g.* from microcrystal slurries	better than 2 Å; completeness, high redundancy, serial MX / SFX, ED	Made-to-order homogenous slurries, bright micro-focus beams
High temporal resolution Fast and/or slow reactions, homogenous samples, initiation	pump-probe; light; mixing; gas; fs – XFELs; μs – Synchrotrons; ms – cryo-EM/ED via freeze trapping	Caged compounds; sample handling; triggering methods; appropriate quantum yields

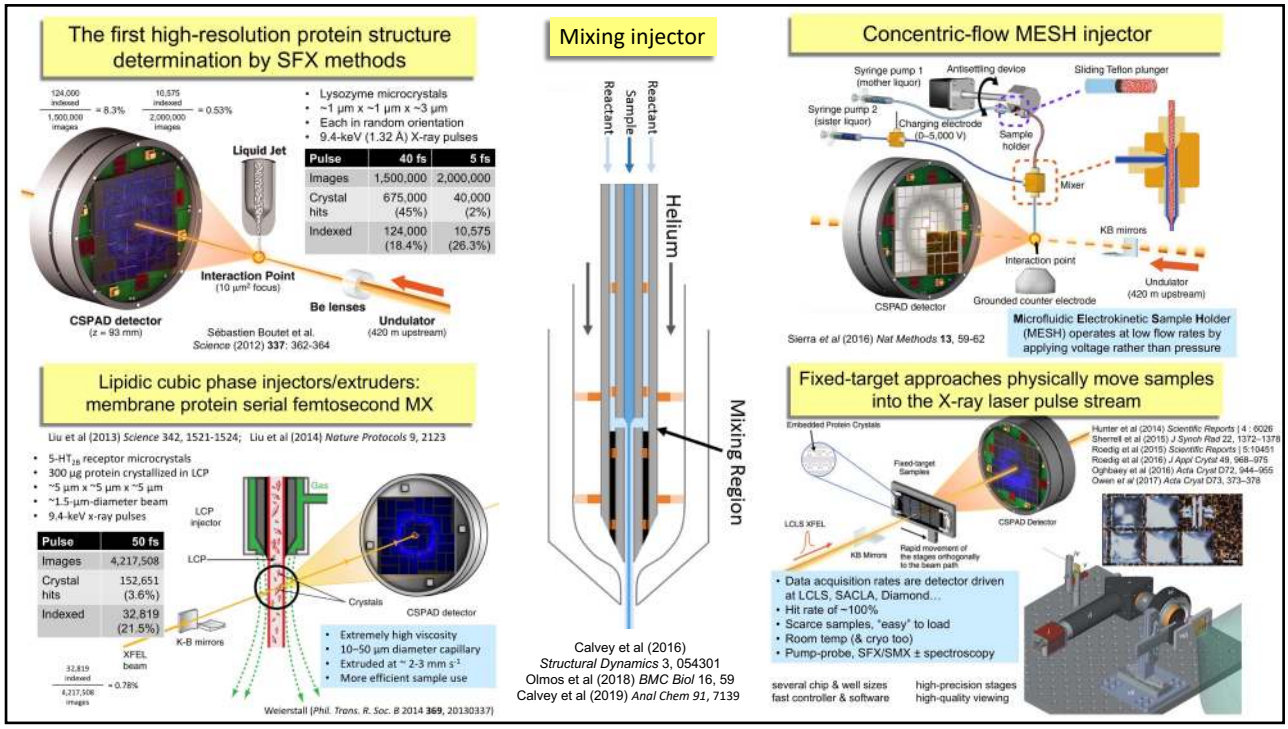
Barends et al (**2022**) *Nature Reviews Methods Primers* 2, 59
Schulz et al (**2022**) *Acta Cryst D 78*, 14-29
Gorel et al (**2021**) *IUCrJ 8*, 532-543
Hough & Owen (**2021**) *Curr Opin Struct Biol 71*, 232-238
Kupitz et al (**2020**) *Crystals 10*, 251
Bernstein (**2020**) *Struct Dyn 7*, 014302
Pearson & Mehrabi (**2020**) *Curr Opin Struct Biol 65*, 168-174
Orville, A. M. (**2020**) *Curr Opin Struct Biol 65*, 193-208

20

Dynamic structural biology → observing structure & function

An ideal experiment…	How & Where ?	What do we need ?
One or more correlated methods tr-SMX/SFX ± tr-XES ± optical ± other X-ray spectroscopies	Integrated instrumentation, Synchrotrons, XFELs, (EM &/or MeV-ED facilities)	Data tracking, analysis, feedback, deep datasets (Δt and ground-state); joint refinement strategies
Under physiological conditions Temp, pressure, pH, salt …	pump-probe, illumination, reagent mixing; equilibration Δt range	Catalytic rates in crystal slurries; efficient sample delivery methods
High spatial resolution *e.g.* from microcrystal slurries	better than 2 Å; completeness, high redundancy, serial MX / SFX, ED	Made-to-order homogenous slurries, bright micro-focus beams
High temporal resolution Fast and/or slow reactions, homogenous samples, initiation	pump-probe; light; mixing; gas; fs – XFELs; μs – Synchrotrons; ms – cryo-EM/ED via freeze trapping	Caged compounds; sample handling; triggering methods; appropriate quantum yields
Element-specific reporter(s) help identify / verify critical reaction intermediates (*e.g.* Fe(IV)=O)	Native (transition metals), post-translational modification(s); multiple beamlines / off-line	Integrated / correlated data streams; joint refinement strategies
Easy access; we must repeat critical observations	Synchrotrons (more plentiful) XFELs (very scarce)	Routine access; similar strategies &/or equipment
All data is curated in database(s) and released upon publication	International standards; metadata from different beamlines / formats	PDB should accept, verify, curate, release correlated datasets

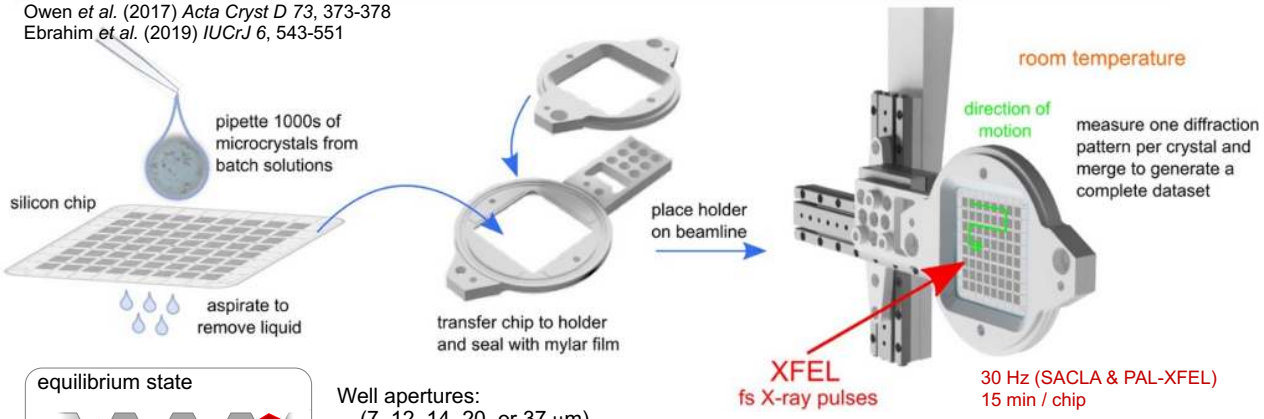
21

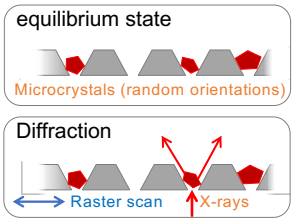


22

Serial crystallography by raster scanning fixed targets

Owen *et al.* (2017) *Acta Cryst D 73*, 373-378
Ebrahim *et al.* (2019) *IUCrJ 6*, 543-551





Well apertures:
(7, 12, 14, 20, or 37 μm)
64 blocks / chip
400 wells / block
25,600 wells / chip
1+ still image / well
Hit ratios ~ 30% to 90+ %

Synchrotron
typically, μs - ms exposures

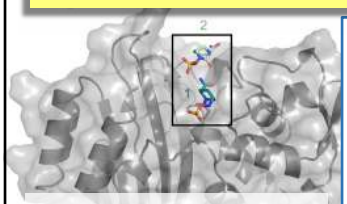
30 Hz (SACLA & PAL-XFEL)
15 min / chip

55 Hz (Diamond I24)
7.8 min / chip
(10 exposures / well → ~50 min.)

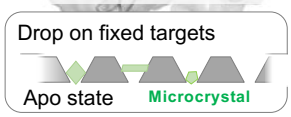
Lucic *et al.* (2021) *J Biol Inorg Chem* 26, 743-761
Rabe *et al.* (2020) *IUCrJ* 7, 901-912

23

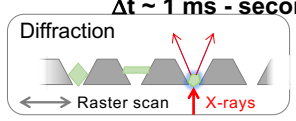
Time-resolved serial crystallography of β-lactamase CTX-M-15 + avibactam

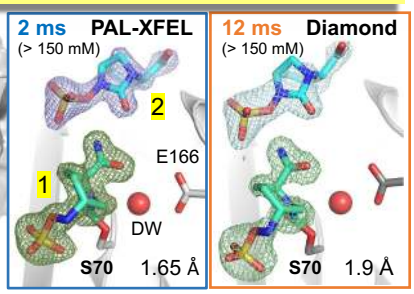


Fixed targets: Diamond I24, all synchrotrons, & XFELs

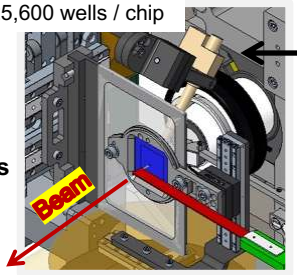


Δt ~ 1 ms - seconds





Raster scan
25,600 wells / chip



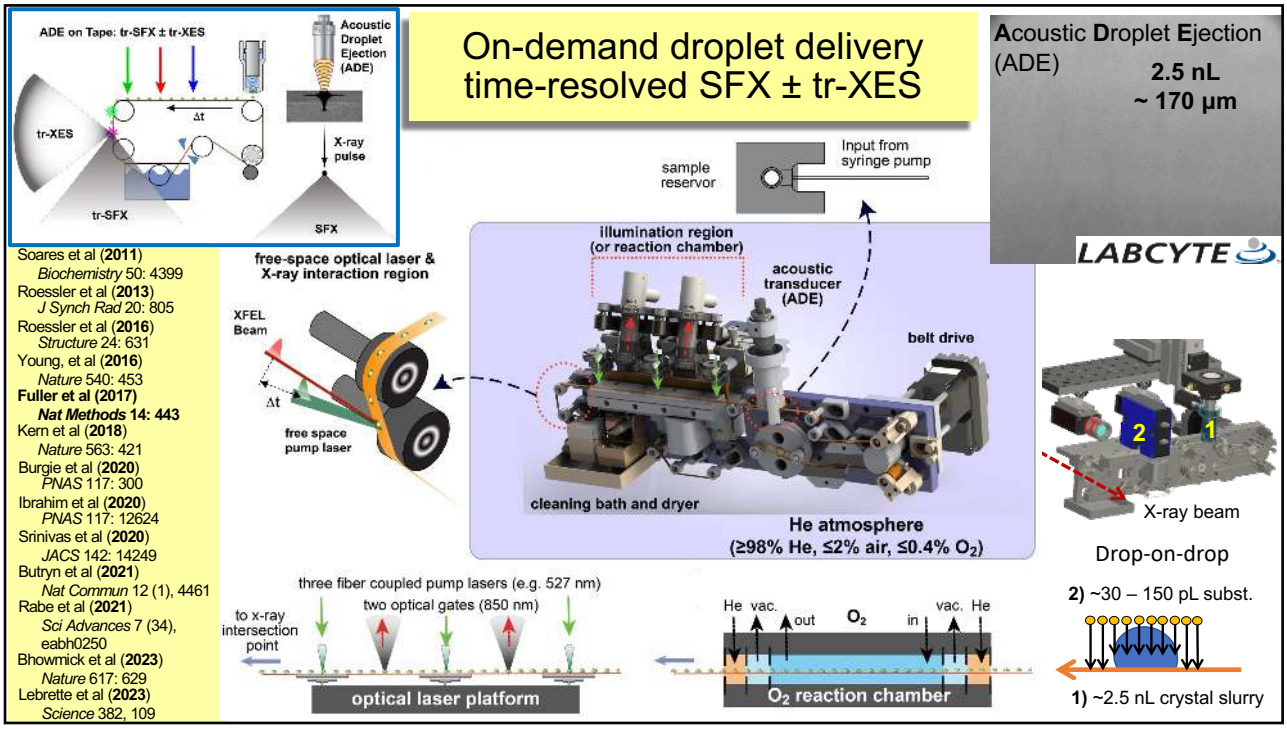
Ligand microdroplet injector
(microdrop Technologies, Norderstedt Germany)

Inspired by:
Mehrabi, P. *et al.* Liquid application method for time-resolved analyses by serial synchrotron crystallography.
Nat Methods **16**, 979-982 (2019)

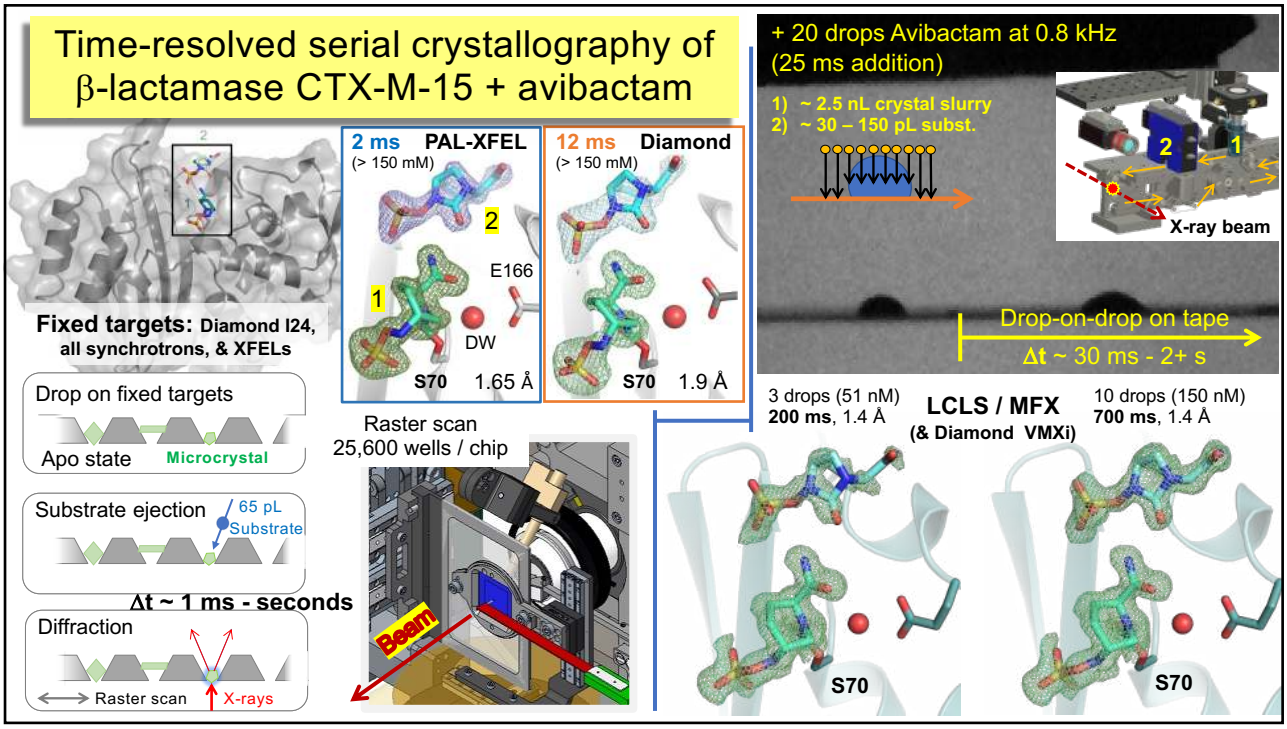
However, since wells are not completely sealed, very important controls must also be collected to be sure ligand does not diffuse to adjacent wells …

→ May compromise Δt data point(s)

24


On-demand droplet delivery
time-resolved SFX ± tr-XES
ADE on Tape: tr-SFX ± tr-XES
Acoustic Droplet Ejection (ADE)
tr-XES
tr-SFX
X-ray pulse
SFX
Acoustic Droplet Ejection (ADE)
2.5 nL
~ 170 µm
LABCYTE
sample reservoir
Input from syringe pump
illumination region (or reaction chamber)
acoustic transducer (ADE)
belt drive
cleaning bath and dryer
He atmosphere
(≥98% He, ≤2% air, ≤0.4% O2)
free-space optical laser & X-ray interaction region
XFEL Beam
free space pump laser
Soares et al (2011) Biochemistry 50: 4399
Roessler et al (2013) J Synch Rad 20: 805
Roessler et al (2016) Structure 24: 631
Young, et al (2016) Nature 540: 453
Fuller et al (2017) Nat Methods 14: 443
Kern et al (2018) Nature 563: 421
Burgie et al (2020) PNAS 117: 300
Ibrahim et al (2020) PNAS 117: 12624
Srinivas et al (2020) JACS 142: 14249
Butryn et al (2021) Nat Commun 12 (1), 4461
Rabe et al (2021) Sci Advances 7 (34), eabh0250
Bhowmick et al (2023) Nature 617: 629
Lebrette et al (2023) Science 382, 109
2
1
X-ray beam
Drop-on-drop
2) ~30 – 150 pL subst.
1) ~2.5 nL crystal slurry
three fiber coupled pump lasers (e.g. 527 nm)
two optical gates (850 nm)
to x-ray intersection point
optical laser platform
He vac.
out
O2
in
vac. He
O2 reaction chamber

25


Time-resolved serial crystallography of β-lactamase CTX-M-15 + avibactam
+ 20 drops Avibactam at 0.8 kHz
(25 ms addition)
1) ~ 2.5 nL crystal slurry
2) ~ 30 – 150 pL subst.
2
1
X-ray beam
Drop-on-drop on tape
Δt ~ 30 ms - 2+ s
2 ms PAL-XFEL
(> 150 mM)
12 ms Diamond
(> 150 mM)
E166
DW
S70 1.65 Å
S70 1.9 Å
Fixed targets: Diamond I24, all synchrotrons, & XFELs
Drop on fixed targets
Apo state
Microcrystal
Substrate ejection
65 pL
Substrate
Δt ~ 1 ms - seconds
Diffraction
Raster scan
X-rays
Raster scan
25,600 wells / chip
Beam
3 drops (51 nM)
200 ms, 1.4 Å
LCLS / MFX
(& Diamond VMXi)
10 drops (150 nM)
700 ms, 1.4 Å
S70
S70

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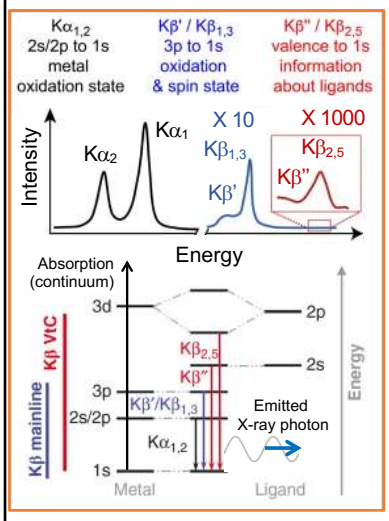
X-ray Emission Spectroscopy

Diamond project S0714



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

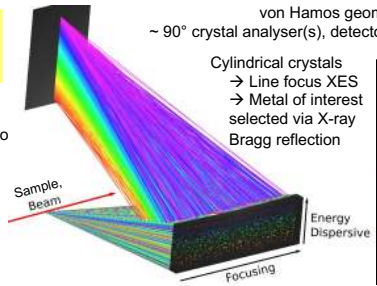
Multiple crystals → samples larger spherical solid angle, better signal to noise ratio and higher sensitivity

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

XES from mixed metal systems

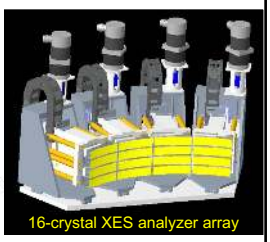
2022 scope:
$K_{\alpha1,2}$, $K_{\beta1,3}$, and $K_{\beta2,5}$ XES from **Fe** and **Cu** metalloproteins

Mature scope:
Kα & Kβ XES from Mn, Co, Ni, Mo, V, W, *etc.* metalloproteins



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

Cylindrical crystals
→ Line focus XES
→ Metal of interest selected via X-ray Bragg reflection


16-crystal XES analyzer array

JIB Periodic Table Last 50 years

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	*Ln*	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	*An*	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Nh	Fl	Mc	Lv	Ts	Og

Lanthanoids:	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Actinoids:	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

	>500	articles in JIB since 1973
	300-500	articles in JIB since 1973
	200-300	articles in JIB since 1973
	100-200	articles in JIB since 1973
	10-100	articles in JIB since 1973
	<10	articles in JIB since 1973
		not considered
		radioactive or inert gas

Reedijk, J. (2020) Fifty years of inorganic biochemistry: Developments, trends, highlights, impact and citations *J Inorg Biochem 212*, 111230

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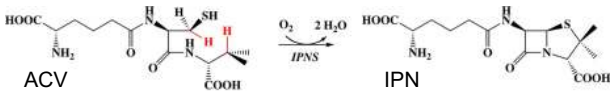
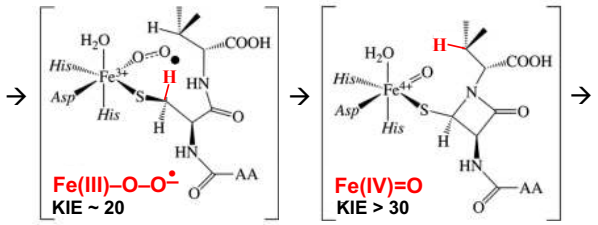
Isopenicillin N synthase (IPNS) & the *"Holy Grail"*

IPNS•Fe(II)•ACV + O_2

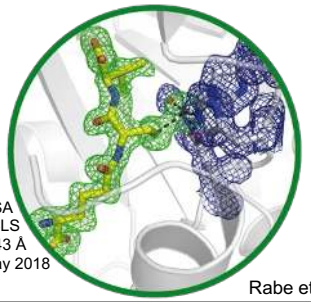


- 2 C–H bonds, large ^{2}H kinetic isotope effects (KIE)
- Fe(III)-superoxo & high-spin **Fe(IV)=O intermediates**
- Representative of large families of Fe oxidases
- Correlated tr-SFX and tr-XES (at LCLS, SACLA, PAL-XFEL, SwissFEL, European XFEL, Diamond Light Source)

Fe(IV)=O is critical throughout aerobic life ***("Holy grail")***
tr-SFX ± tr-XES with ADE on tape and mixing GDVN methods



IPNS•Fe(II)•ACV

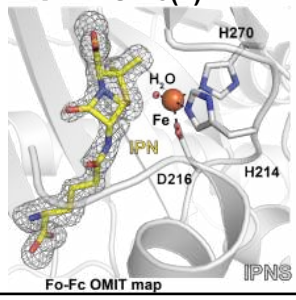


USA
LCLS
1.43 Å
May 2018

+ O_2 →

"Chemistry happens"
k_{cat} ~ 4.6 s^{-1} (in solution)
… can we watch it?

→ IPNS•Fe(II)•IPN



Fo-Fc OMIT map

Japan
SACLA
Nov
2017

Rabe et al; (2021) *Science Advances* **7**, eabh0250

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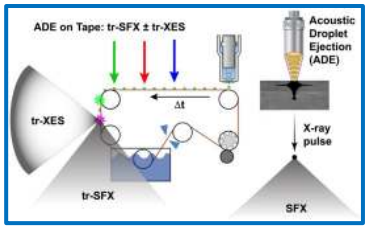
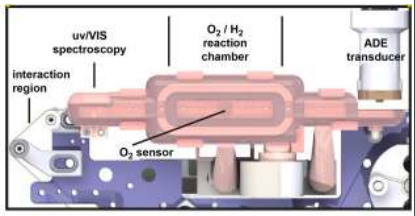
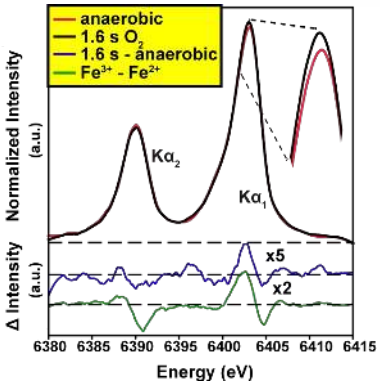
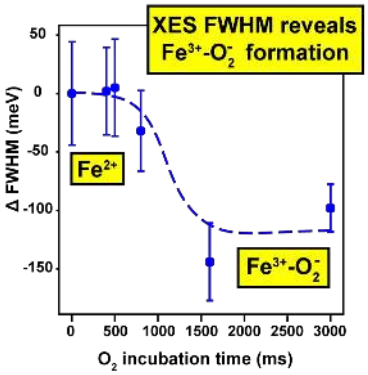
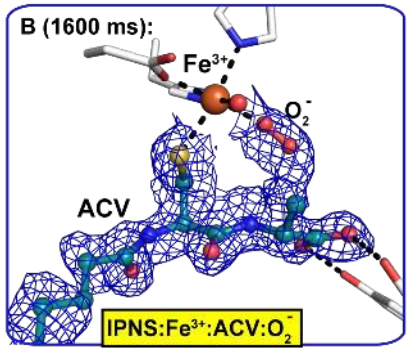


Mechanistic insights from time-resolved SFX correlated with time-resolved X-ray emission spectroscopy









Fuller *et al.* (2017) *Nat Methods 14*, 443-449
Butryn *et al.* (2021) *Nat Commun 12*, 4461
Rabe *et al.* (2021) *Science Advances* **7**, eabh0250

Spectroscopic analyses *in crystallo* supports formation of an Fe(III)-O–O$^-$ species early in the IPNS-ACV-O_2 reaction

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Dynamic Structural Biology

Diamond project S0713:
- On-demand sample delivery
- Three reaction initiation strategies
- Anaerobic conditions

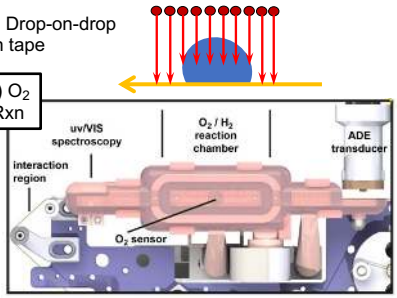
Diamond project S0714:
- X-ray emission spectroscopy (XES)
- Correlated with serial crystallography

Max. delivery (Hz)	Drop volume	Drop diam.	Pancake height
~ 150	3 nL	180 µm	140 µm
~ 150	1 nL	124 µm	100 µm
30 k	150 pL	66 µm	52 µm
30 k	100 pL	60 µm	45 µm
30 k	75 pL	50 µm	41 µm
30 k	50 pL	45 µm	36 µm
30 - 50 k	30 pL	40 µm	30 µm

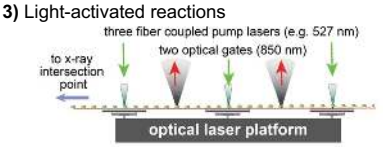
Blue outline = Acoustic droplet ejection (ADE)
Red outline = Piezoelectric (*e.g.* PolyPico)

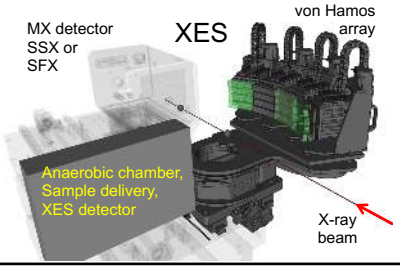
1) Drop-on-drop on tape

2) O_2 Rxn



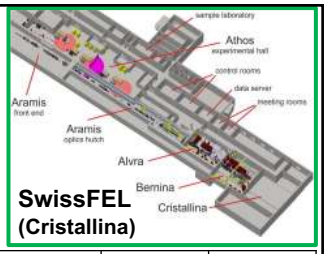
3) Light-activated reactions





Time-resolved studies at

Diamond Light Source (VMXi) ⇄ **SwissFEL (Cristallina)**



von Hamos analyser crystals	Energy (keV)	Status
4x Si(4,4,4) $Kα_{1,2}$ XES of **Cu** enzymes	8.048 8.028	Commission
16x Si(5,5,3) $Kβ_{1,3}$ XES of **Cu** enzymes	8.905	Commission
4x Ge(4,4,0) $Kα_{1,2}$ XES of **Fe** enzymes	6.404 6.391	Commission
16x Ge(6,2,0) $Kβ_{1,3}$ XES of **Fe** enzymes	7.058	Commission
4x $LiNbO_3$(4,2,2) $Kα_{1,2}$ XES of **Ni** enzymes	7.478 7.461	Ordered
4x Ge(3,3,3) $Kα_{1,2}$ XES of **Mn** enzymes	5.899 5.888	Phase 3
16x Si(4,4,0) $Kβ_{1,3}$ XES of **Mn** enzymes	6.490	Phase 3

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