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MX groups:: Swiss Light Source :: Paul Scherrer Institut

MX beamline upgrade at SLS 2.0

March 1st 2022

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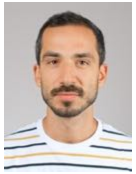
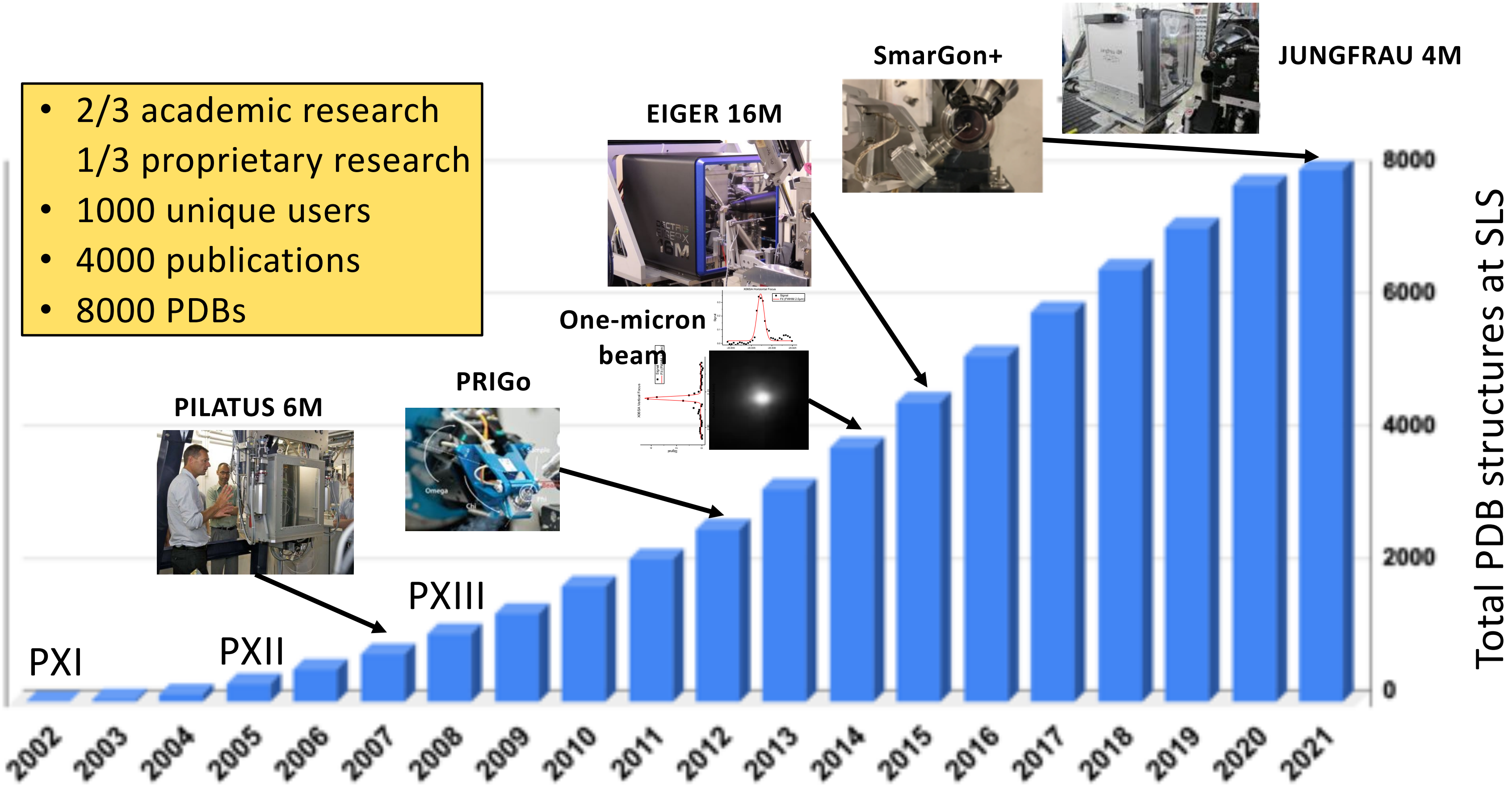


Deniz Eris

PAUL SCHERRER INSTITUT PSI

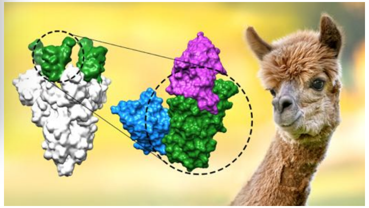
SLS MX Beamlines: 20 years

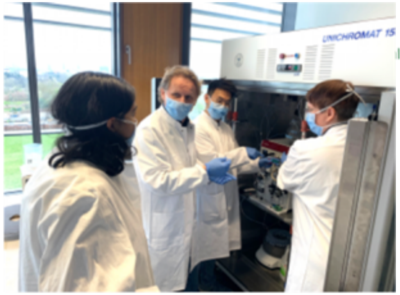
- 2/3 academic research
  1/3 proprietary research
- 1000 unique users
- 4000 publications
- 8000 PDBs

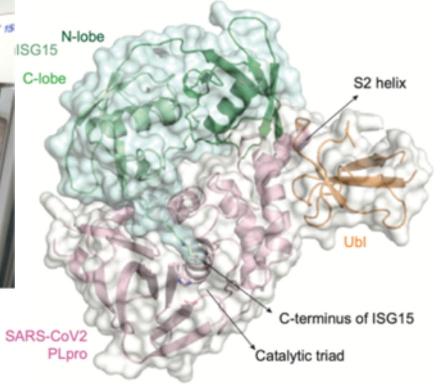


COVID-19, and COVID-19 Related Research

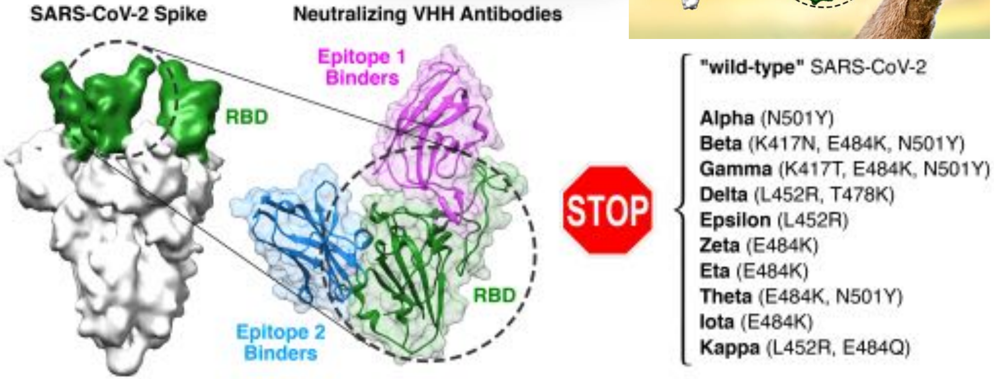
- Continuous user operation through the pandemic
- 100% remote mode since 2020
- > 50’000 sample mounts in 2021
- SLS Special call for COVID-19 research







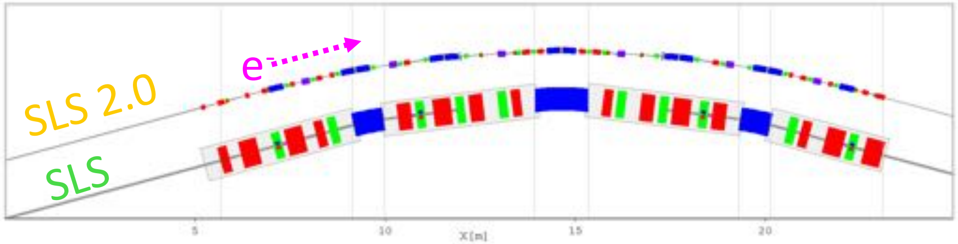
Papain-like protease regulates SARS-CoV-2 viral spread and innate immunity.
Ivan Dikic / University Frankfurt, ***Nature*** (2020)
Four months from X-ray data collection to publication



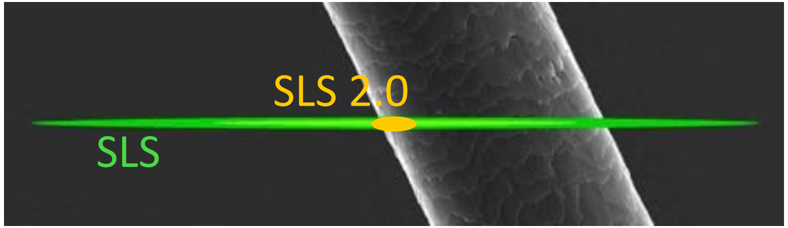
Nanobodies (blue and magenta) bind to the receptor-binding domain (green) of the coronavirus spike protein (grey), thus preventing infection with SARS-CoV-2 and its variants.
Thomas Güttler / MPI for Biophysical Chemistry, ***EMBO J*** (2021)

SLS 2.0 and MX 2.0

SLS 2.0 – a next generation synchrotron **source**



SLS 2.0 lattice design: a 7-bend achromat arc



Electron beam cross section in comparison to human hair (Andreas Streun)

MX 2.0 – next generation MX **beamlines**

2000 PXI – X06SA
2004 PXII – X10SA
2007 PXIII – X06DA

A testbed for new beamlines at SLS 2.0

2022 PXIII – X06DA
2024 PXII – X10SA
2025 PXI – X06SA

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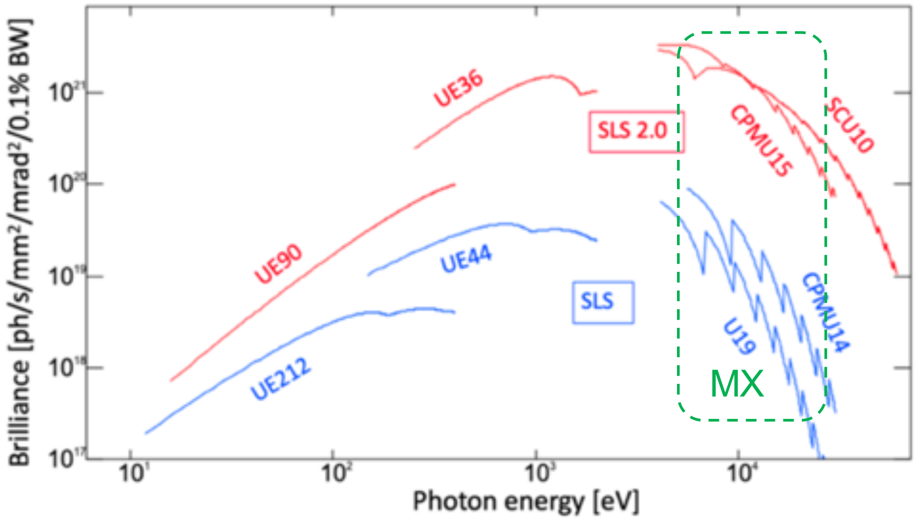
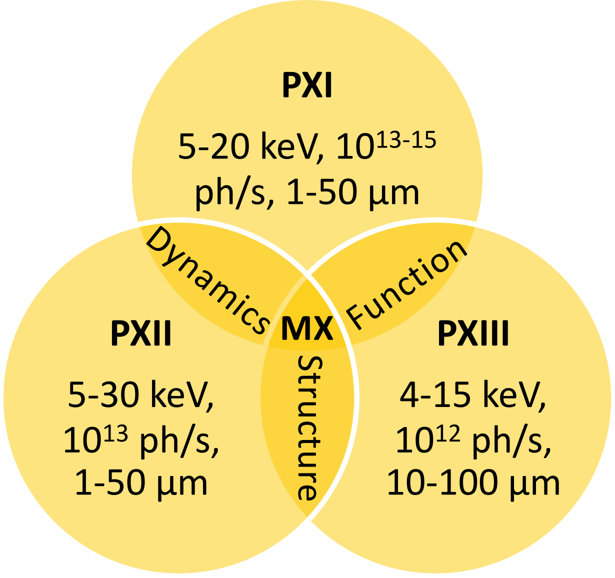
SLS 2.0 : Source Brilliance Improvement

- SLS 2.0 2.7 GeV machine will increase source brilliance up to 30 times
- PXI – ”discovery” beamline in the new era of structural biology
- PXII – micro-focus undulator source for high-throughput and serial MX
- PXIII – robust micro-beam bend magnet source for autonomous MX





Three MX beamlines covering a wide range of applications

X-ray Optics Design

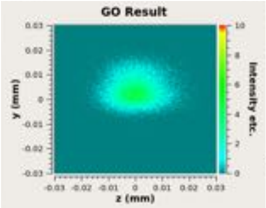
FWHM
18×16 µm
1×0.3 mrad

PXIII
Routine MX

DCCM (4-15 keV)

KB (4.5 mrad)
~2:1 total

15×10 µm (divergence limited)
defocus up to 100 µm
1.5×0.6 mrad
10^{12} photon/sec



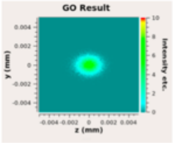
FWHM
47×10 µm
21×12 µrad

PXII
Microfocus MX

DCM/DMM (5-30 keV)

KB (3 mrad)
~20:1

2×1 µm (slope-error limited)
defocus up to 50 µm
0.8×0.4 mrad
10^{13} photon/sec



2 – 10 µm in hori. (geom. optics limited)
1 – 4 µm in vert. (slope-error limited)
0.1 – 0.5 mrad
10^{13-15} photon/sec

FWHM
47×10 µm
21×12 µrad

PXI
Versatile X-ray structural biology

DCM/DMM
(5-30 keV)

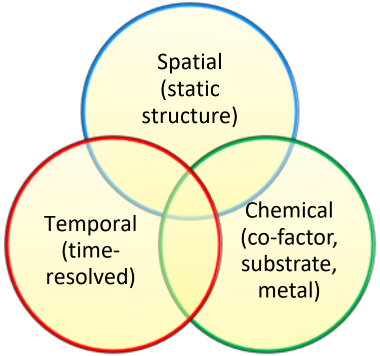
KB (3 mrad)
20:1 – 5:1

Detector rail

Diffractometer rail

Flight tube

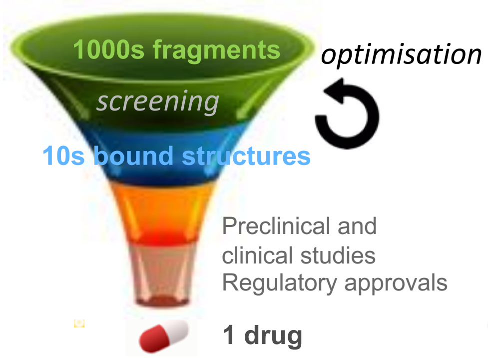
Lasers/spectroscopy bench



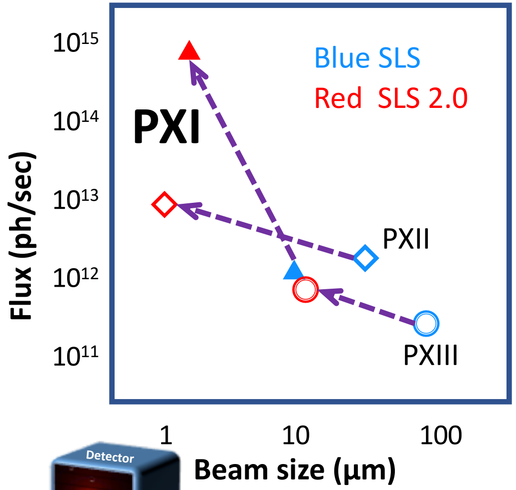
Scientific Benefits of the SLS 2.0 for PX

- **PXI and PXII (3m undulator)**: 10-100× more flux and 10× more parallel beam
- **PXIII (2T bending magnet)** will reach a comparable performance of undulator beamlines at SLS

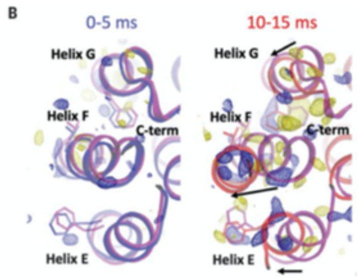
Higher throughput in structure-based drug screening



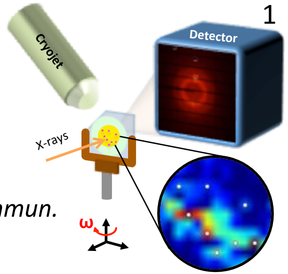
Mühlethaler *et al*, *Angew. Chem.* (2021)



From static structure to movie with higher time-resolution (ms to µs)



Weinert *et al*, *Science* (2019)
Mous *et al*, *Science* (2022)

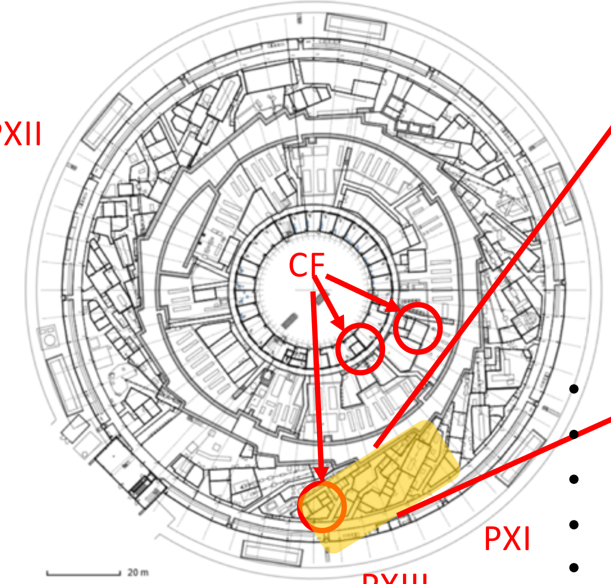


Huang *et al*, *Commun. Biol.* (2018)

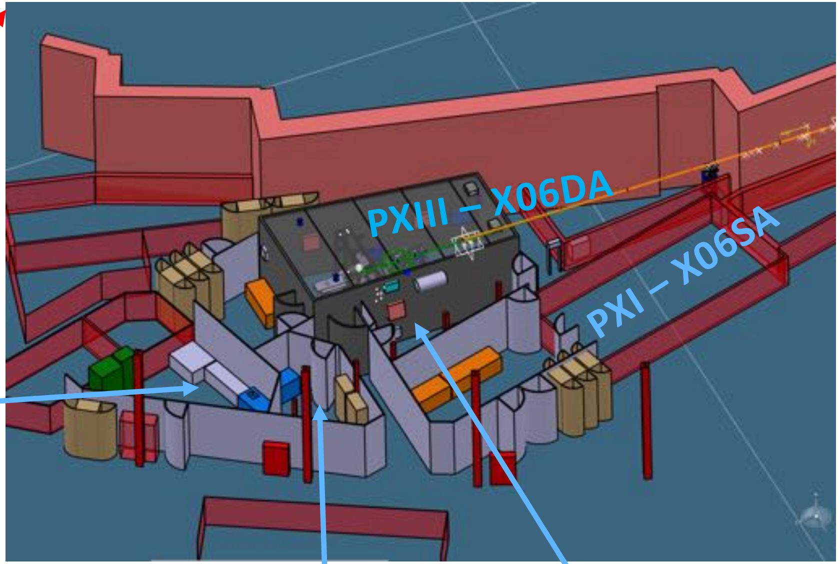
Serial synchrotron crystallography with micron-sized crystals at both cryo- and room-temperature

Affiliated Labs and Crystallization Facility

- Beamline labs purposely rebuilt to support experiments (e.g. light-sensitive sample)
- On-site crystallization facility (CF)
- Dedicated facility for FFCS
- Support on-site sample preparation and delivery (e.g. room-temperature)



- RockImager RT
- Mosquito RT
- Gryphon
- ECHO
- Shifter



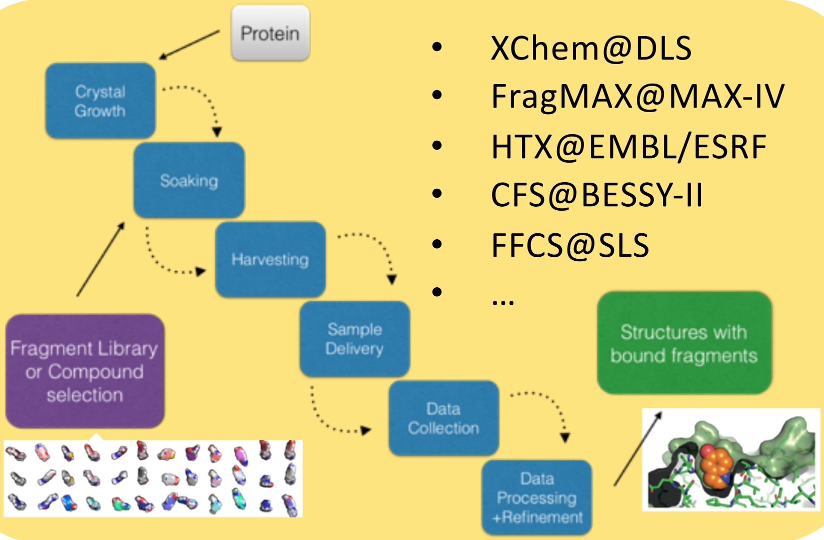
Scientific Cases

- High-throughput crystallography – fragment-based screening
- Multi-temperature crystallography – structural dynamics and mechanism
- Serial synchrotron crystallography – routine method for 10 µm crystals
- Multi-dimensional crystallography – spectroscopy
- Time-resolved crystallography – molecular movie
- Chemical crystallography – into powders

Fragment Based Screening with X-Ray Crystallography

Cryogenic MX (May Sharpe/Günter Fritz talk)

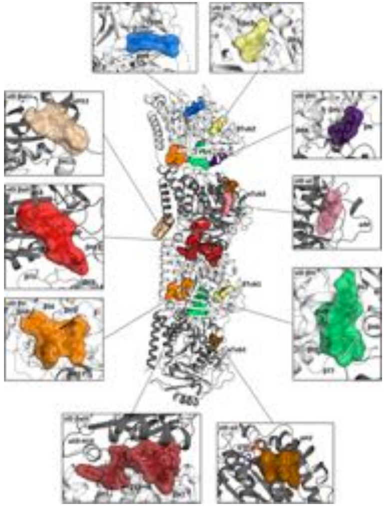
+ routine screening of hundreds fragments
+ sensitive in detecting weak binders
- could suppress heterogeneity of protein conformation and fragment binding mode
- non-physiological temperature



- XChem@DLS
- FragMAX@MAX-IV
- HTX@EMBL/ESRF
- CFS@BESSY-II
- FFCS@SLS
- …

Kaminski J. *et al, Acta Cryst. D in press*

X-ray fragment based screening of tubulin (PSI)



Mühlethaler T. *et al,*
Angew. Chem. Int. Ed. (2021)

FBS enabled discovery of new binding sites for active agents – against cancer, for example – on a vital protein of the cell cytoskeleton. Eleven of the sites hadn't been known before.

Higher throughout at SLS 2.0

Fragment Based Screening with X-Ray Crystallography

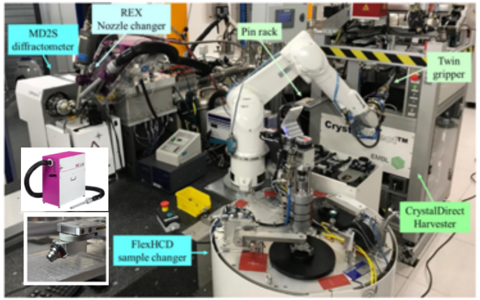
Cryogenic MX

+ routine screening of hundreds fragments
+ sensitive in detecting weak binders
- could suppress heterogeneity of protein conformation and fragment binding mode
- non-physiological temperature

Room-temperature MX

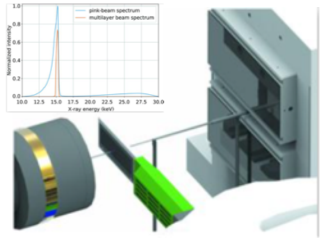
+ access structural heterogeneity
+ relate to RT fragment-based assays
- increase radiation damage
- lower sensitivity to weaker binders
- “manual” sample delivery and low throughput

CrystalDirect-to-Beam@ESRF



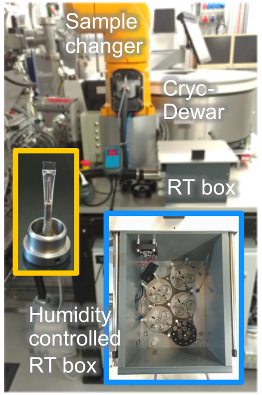
Felisaz F. *et al*, *AIP Conf. Proc.* (2019)

Fixed-target_SSX @DESY/ESRF



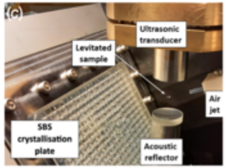
Tolstikova A. *et al*, *IUCrJ* (2019)

RT-automation@SLS



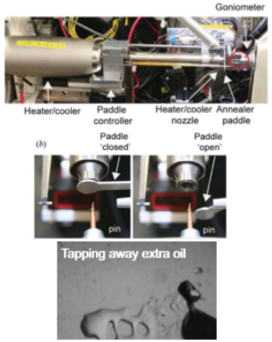
Huang C.-Y. *et al*, *submitted*

AcousticDirect@SLS



Tsujino S. *et al*, *Appl. Phys. Lett.* (2019)

MX-across-RT@SSRL



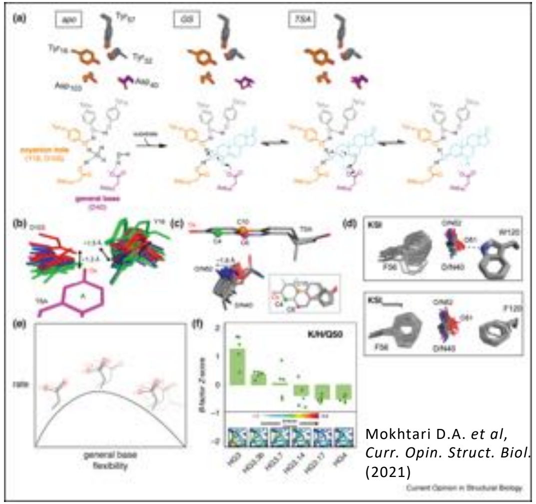
Doukov T. *et al*, *J. Appl. Cryst.* (2020)

Multi-Temperature Crystallography for Function and Mechanism

Multi-temperature MX

- Access to structural heterogeneity at local and global level
- Atomic elucidation of function and mechanism
- Insight of protein dynamics

Fraser J.S. *et al*, *PNAS* (2011)
Keedy D.A. *et al*, *eLife* (2015)
Fischer M. *et al*, *ChemBioChem* (2015)
Keedy D.A. et al, eLife (2018)
Keedy D.A. *et al*, *Acta Cryst. D* (2019)
Broom A. *et al*, *Nat. Commun.* (2020)
Yabukarski F. *et al*, *PNAS* (2020)

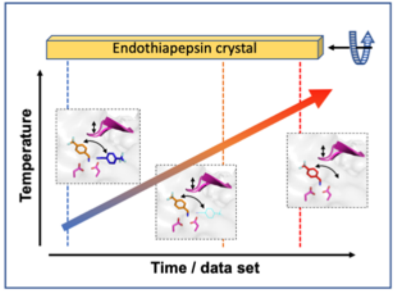


Mokhtari D.A. *et al*, *Curr. Opin. Struct. Biol.* (2021)

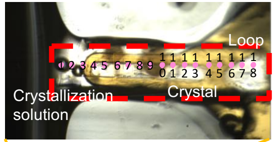
Ensemble crystallography provides mechanistic insights toward enzyme catalysis and engineering

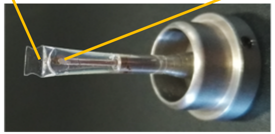
"Temperature-resolved" MX

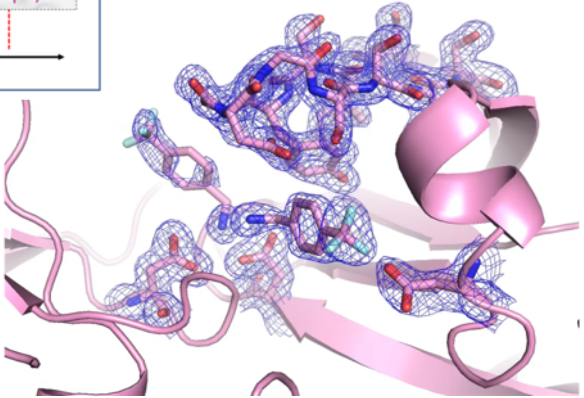
- Observe protein conformational change
- Record alternative ligand binding mode
- Access to non-ground state



- ✓ Cryostream ramped up temperature from 275 to 305 K in 10 min
- ✓ 14 X-ray diffraction data sets were collected from one crystal in a helical manner
- ✓ Automated RT sample mounting at X10SA-PXII, SLS







Development for SLS 2.0

Huang C.-Y. *et al*, *submitted*

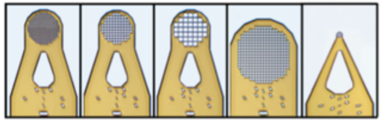
Automated Serial Synchrotron Crystallography (SSX)

Serial synchrotron crystallography

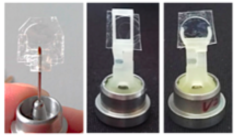
- A routine method for 10 µm crystals at cryo and RT
- Sample delivery with loop, mesh, and chip
- Fast gridscan and serial data collection automation
- Data processing and merging pipeline

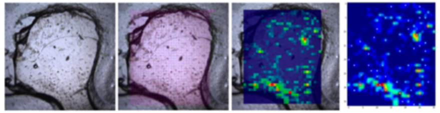
SSX at next generation synchrotrons

- One-micron focused beam – PXI, PXII @SLS 2.0
- Higher X-ray energy (20-30 keV) – PXII @SLS 2.0
- kHz data collection with next generation detectors

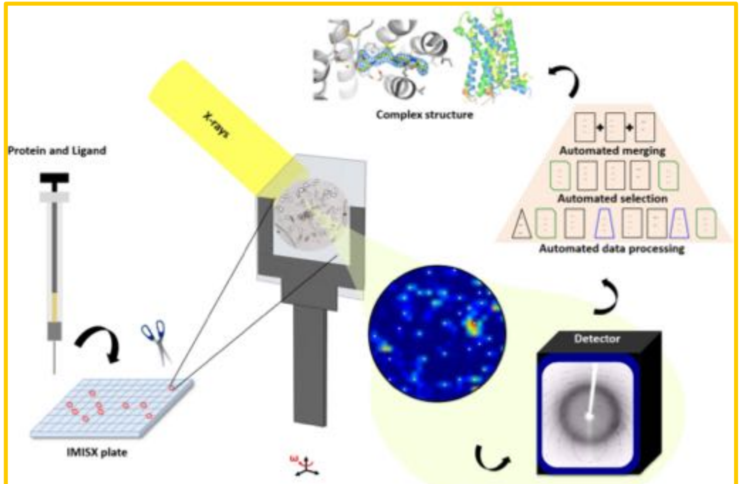


Mitiger mesh loops (https://www.mitegen.com/)

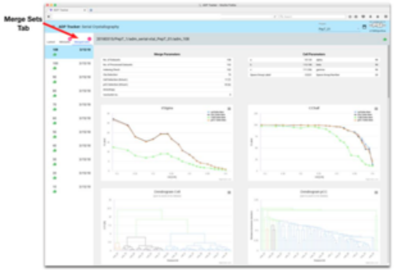




Collaboration with Martin Caffrey



Cheng R. *et al, FEBS Journal* (2019)



Data selection methods

- *ISa*
- *Unit cell cluster*
- *Pairwise correlation*
- *Isocluster*
- $\Delta CC_{1/2}$

Basu S., *et al, Acta Cryst. D* (2018)
Assmann G. *et al, Acta Cryst. D* (2020)

Collaboration with Kay Diederichs

Multi-Dimensional Crystallography
(Spatial, chemical, temporal)

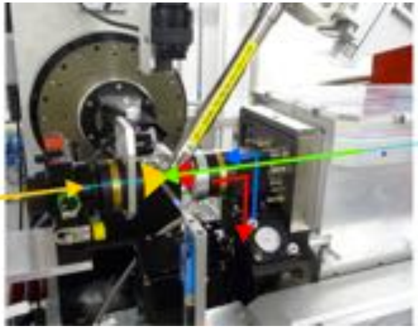
Multi-dimensional MX

- X-ray cryogenic “single” structure
- X-ray room-temperature structure ensembles
- X-ray multi-temperature structures
- Redox state of metals and chemical state of cofactors by *in crystallo* spectroscopy
- Time-resolved study

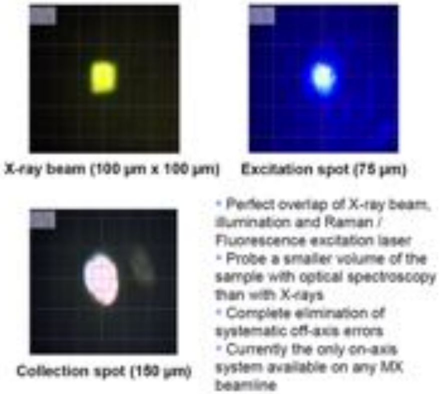
- UV/Vis absorption spectroscopy
  - colored co-factors, metalloproteins
- Fluorescence spectroscopy
  - co-factors, substrates, redox state of metals
- Raman spectroscopy
  - chemical species
- Preparation of time-resolved MX
  - Check and track light-triggered structure changes
  - Check crystal diffraction upon trigger illumination

PXII on-line multi-mode micro-spectrophotometer

On-axis microscope (blue)
X-rays (green)
UV/Vis (yellow)
Raman and fluorescence (red)



Pompidor G. *et al, JSR* (2013)

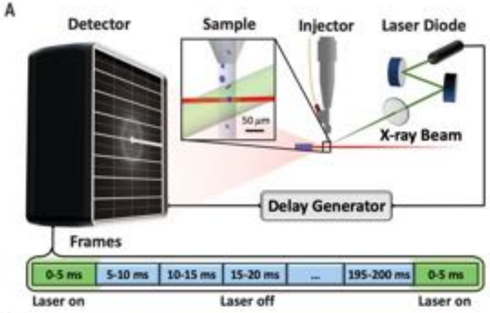


PXI implementation @ SLS 2.0 to complement time-resolved MX

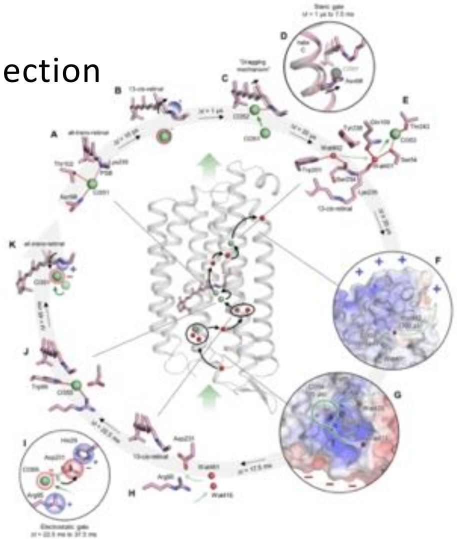
Time-Resolved Synchrotron Crystallography (TR-SSX)

TR-SSX with injector at SLS (Sandra Mous talk)

- Complementary to SFX@XFEL
- 5-10 µm X-ray beam
- Laser-diode triggering
- EIGER 200 Hz data collection
- 5 ms time resolution



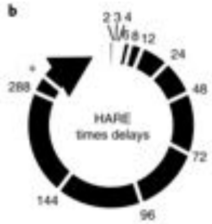
”Proton uptake mechanism in bacteriorhodopsin captured by serial synchrotron” Weinert T. *et al Science* (2019)



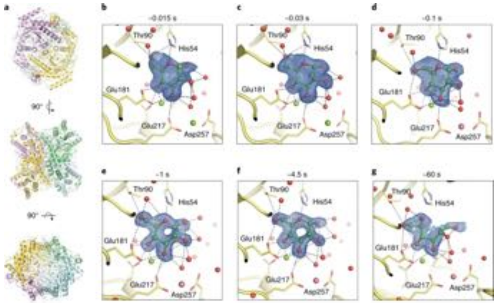
“Dynamics and mechanism of a light-driven chloride pump” Mous S. *et al Science* (2022)

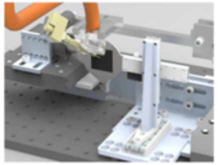
TR-SSX with fixed-target at PETRA-III

- HARE - hit-and-return approach
- LAMA – liquid application method



HARE time delay	Corresponding time point	
-	12×12 chip (ms)	24×24 chip (ms)
2	171	188
3	256.5	282
4	342	376
6	513	564
8	684	752
12	1,026	1,128
24	2,052	2,256
48	4,104	4,512
72	6,156	6,768
96	8,208	9,024
144	12,312	13,536
288	24,624	27,072





LAMA enables the time-resolved analysis of the catalytic activity of xylose isomerase.

“The hit-and-return system enables efficient time-resolved serial synchrotron crystallography” Schulz E. *et al Nat. Methods* (2018)

“Liquid application method for time-resolved analyses by serial synchrotron crystallography” Mehrabi P. *et al Nat. Methods* (2019)

Enabling Advanced Chemical Crystallography

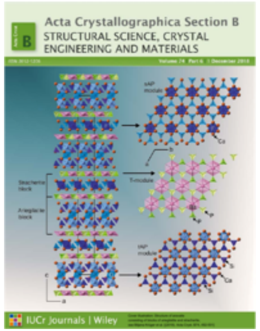
Chemical crystallography

- High-energy, high-flux, micro-beam, large detector, multi-axis goniometer
- Sample changer for both cryo and room-temperature
- Routinely used by industry and academics
- PXII – single crystal structure of drug-like compounds
- PXIII – chemistry, materials science, minerals

Mineralogical crystallography at PXIII

Discover new mineral species with diverse compositions and complex substitutions

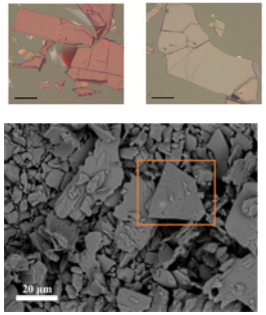
IMA No.	Name	CNMNC approved formula
2020-091	shagamite	$KFe_{11}O_{17}$
2020-073	devilliersite	$Ca_4Ca_2Fe^{3+}_{10}O_4[(Fe^{3+}_{10}Si_2)O_{36}]$
2019-080	gorerite	$CaAlFe^{3+}O_{19}$
2019-068	bennesherite	$Ba_2Fe^{2+}Si_2O_7$
2019-007	gmalimite	$K_6\square Fe^{2+}_{24}S_{27}$
2018-158	kahlenbergite	$KAl_{11}O_{17}$
2018-150	siwaqaite	$Ca_6Al_2(CrO_4)_3(OH)_{12}\cdot 24H_2O$
2018-140	khurayyimite	$Ca_7Zn_4(Si_2O_7)_2(OH)_{10}\cdot 4H_2O$
2018-078	aravaite	$Ba_2Ca_{18}(SiO_4)_6(PO_4)_3(CO_3)F_3O$
2017-049	zoharite	$(Ba,K)_6(Fe,Cu,Ni)_{25}S_{27}$
2017-014	sharyginite	$Ca_3TiFe_2O_8$
2017-010	levantite	$KCa_3Al_2(SiO_4)(Si_2O_7)(PO_4)$
2016-100	ariegilatite	$BaCa_{12}(SiO_4)_4(PO_4)_2F_2O$
2016-098	stracherite	$BaCa_6(SiO_4)_2[(PO_4)(CO_3)]F$



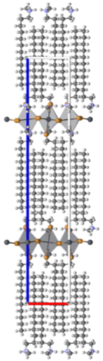
Krüger, B., Krüger H., Kahlenberg V., Institute of Mineralogy & Petrography, University of Innsbruck

Materials research

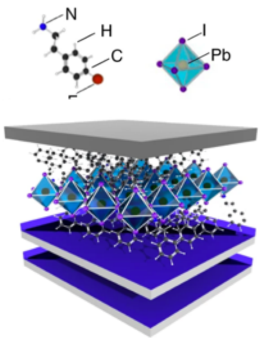
Solar cell application – lighting up the world of hybrid perovskite



Dhanabalan B. *et al.* (2021) **Advanced Materials**



Polimeno L. *et al.* (2021) **Nature Nanotechnology**

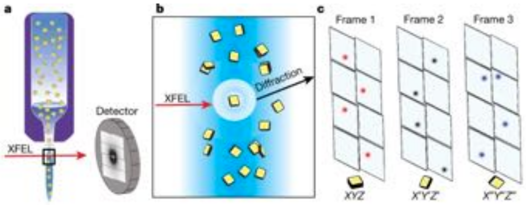


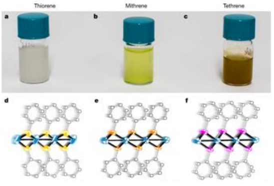
Cinquino M . *et al.* (2021) **Advanced Materials**

Istituto di Cristallografia Consiglio, Nazionale delle Ricerche, Bari, Italy

Chemical crystallography by SFX at LCLS

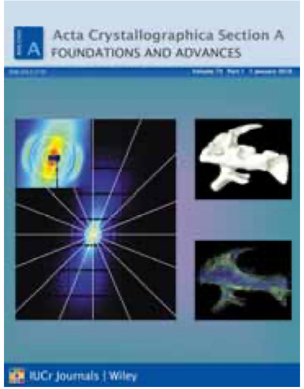
Inorganic-organic hybrid materials





Schriber E. A. *et al.* (2022) **Nature**

Beyond Crystallography: Small Angle X-ray Scattering Tensor Tomography



Marianne Liebi *et al.*, Nature **527**, 349 (2015). DOI: 10.1038/nature16056,
Acta Cryst. **A74**, 12 (2018). DOI: 10.1107/S205327331701614X

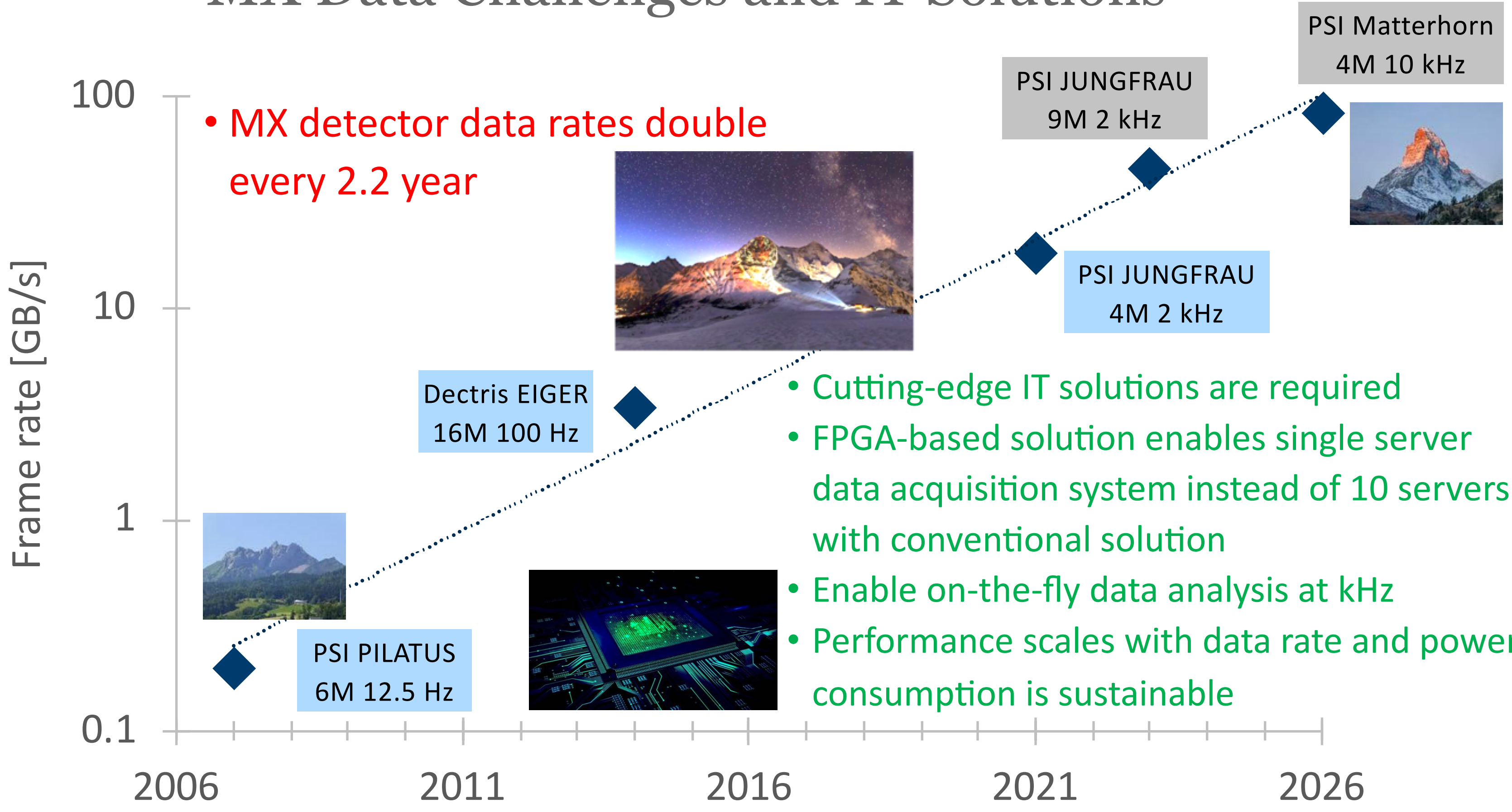
SAXS tensor tomography (SAS TT)

- Multi-axes goniometer
- Cryo conditions
- High flux
- Fast grid-scanning with fast area detector
- Full-azimuth WAXS option

MX and SAS TT: The major difference is the sample-to-detector distance (...).

cSAXS: develop SAS-TT method
Marianne Liebi: drive life science applications
MX: automate SAS-TT for a wider community

MX Data Challenges and IT Solutions



- MX detector data rates double every 2.2 year

- Cutting-edge IT solutions are required
- FPGA-based solution enables single server data acquisition system instead of 10 servers with conventional solution
- Enable on-the-fly data analysis at kHz
- Performance scales with data rate and power consumption is sustainable

"JUNGFRAU detector for brighter x-ray sources: Solutions for IT and data science challenges in macromolecular crystallography"
Leonarski F., *et al, Structural Dynamics* (2020)

PAUL SCHERRER INSTITUT PSI

Timeline and planning

- Staged upgrades in pre-dark period, dark period, and post-dark period
- PXII/PXIII is among priority beamlines, which should resume user operation asap

	2022				2023				2024				2025				2026			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
PXI	User operation							Upgrade I									Upgrade II		User operation	
PXII	User operation							Complete upgrade									Fine-tune		User operation	
PXIII		Upgrade I				User operation		Upgrade II									Fine-tune		User operation	

- PXIII pre-upgrade as a testbed for new technology
- PXIII users measure at PXI/II

SLS 2.0 machine upgrade

ESRF beamtime coverage for beamline partners

Pilot users

Second "dark period" for sources and beamlines

Acknowledgements

Swiss Light Source
MX group
X-ray optics group
Undulator group
LSB/PSD management
SLS-2 project

PSI Grossforschungsanlagen

PSI detector group

PSI Science IT

PSI TR-SFX group

Users and partners of PX beamlines