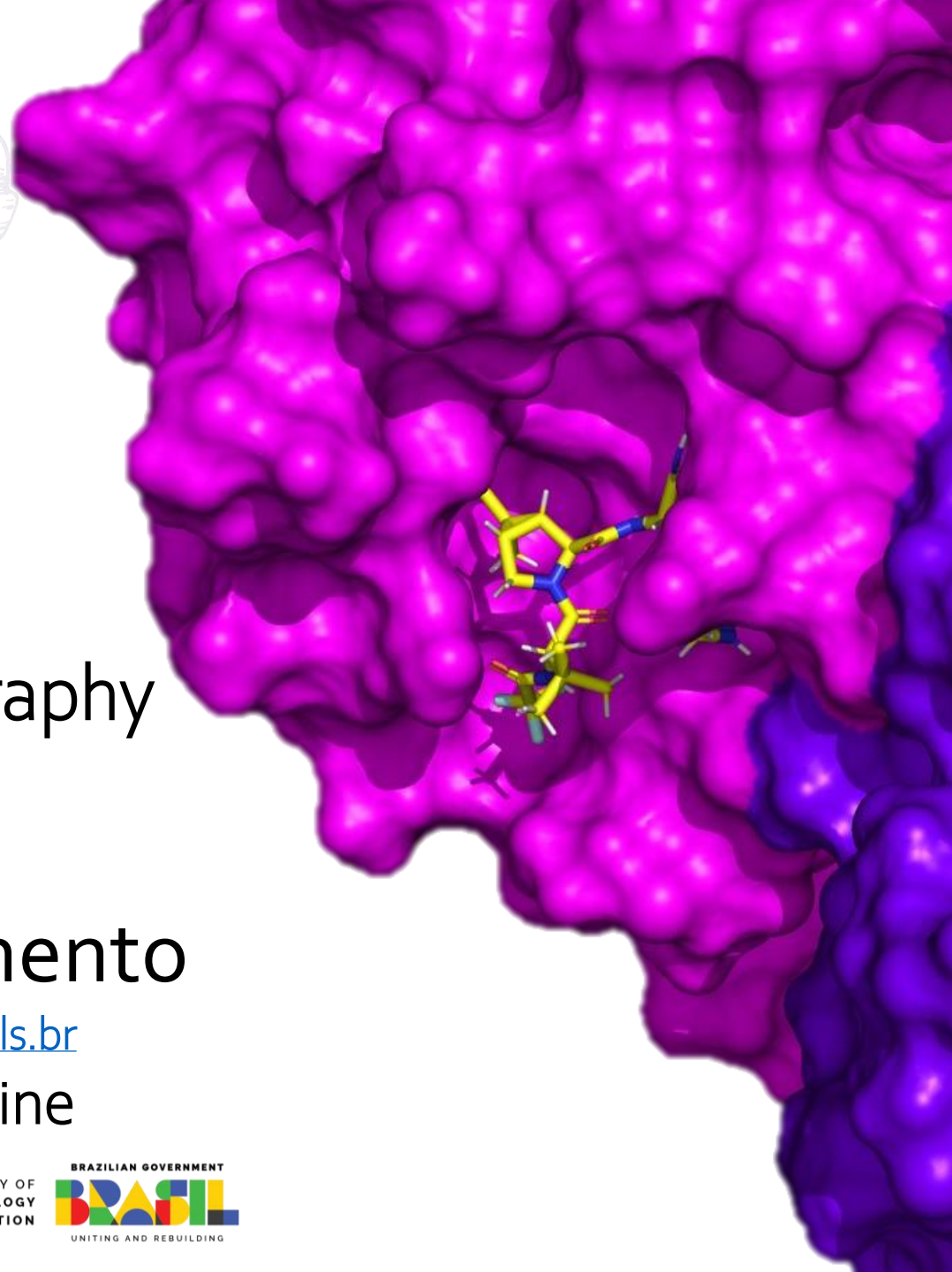
MXCuBE-ISPyB
Joint Meeting
Trieste, Italy / 20-22 November 2024

Elettra Sincrotrone Trieste

MANACÁ@Sirius

macromolecular and chemical crystallography



Andrey Nascimento

andrey.nascimento@lnls.br

MANACÁ beamline

LNLS Brazilian Synchrotron Light Laboratory

CNPEM Brazilian Center for Research in Energy and Materials

MINISTRY OF SCIENCE TECHNOLOGY AND INNOVATION

BRAZILIAN GOVERNMENT BRASIL UNITING AND REBUILDING

Acknowledgment

MANACÁ group

Andrey Nascimento – coordinator

Evandro Araujo – researcher

Igor Maldonado – specialist

Felipe Ramos - dev. analyst

João Rodriguez - dev. analyst

Ana Julia Silva - intern

Pedro Benetton - intern

Ana Beatriz Carvalho - student

All the support teams.

Users!

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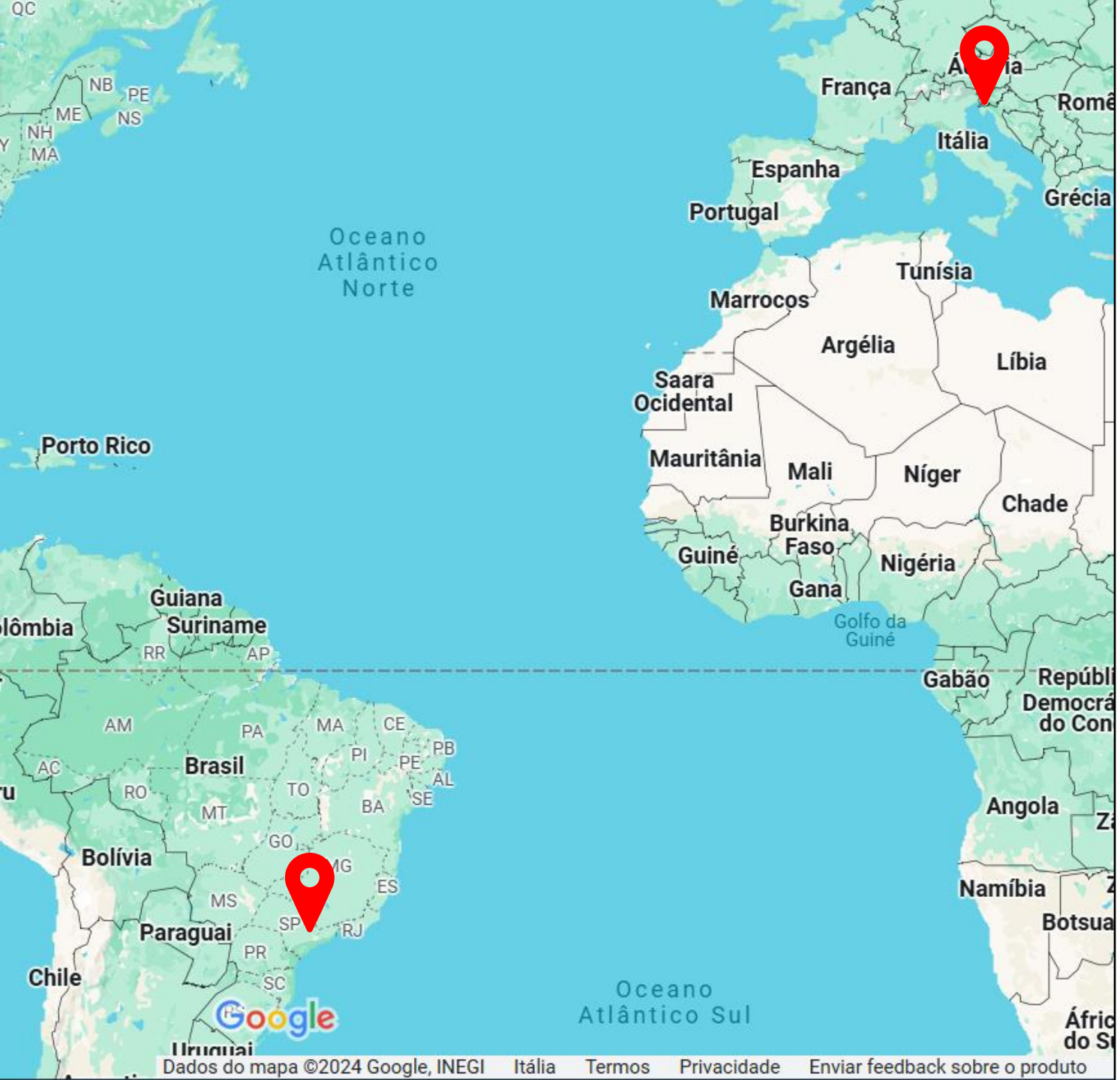
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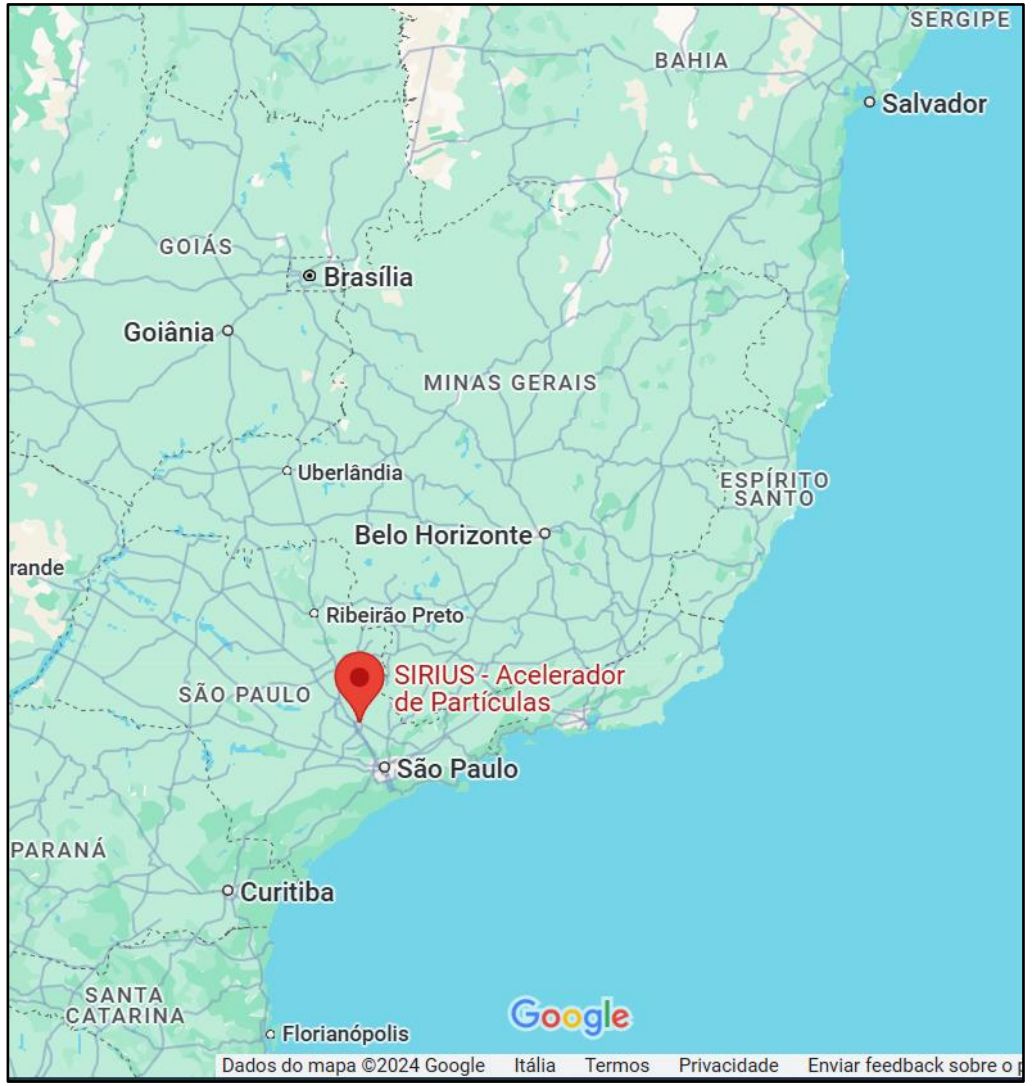
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Uberlândia
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Belo Horizonte
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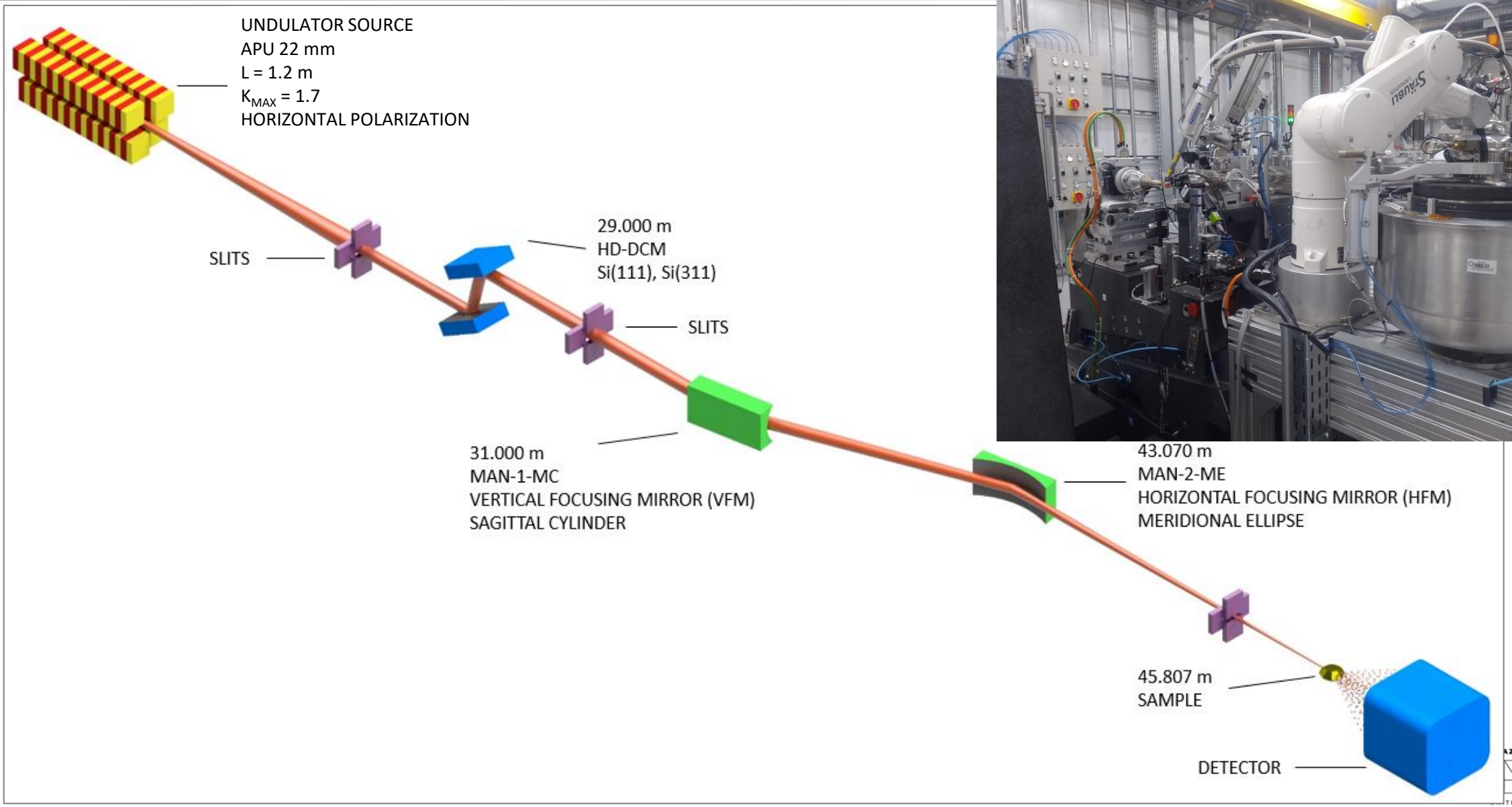
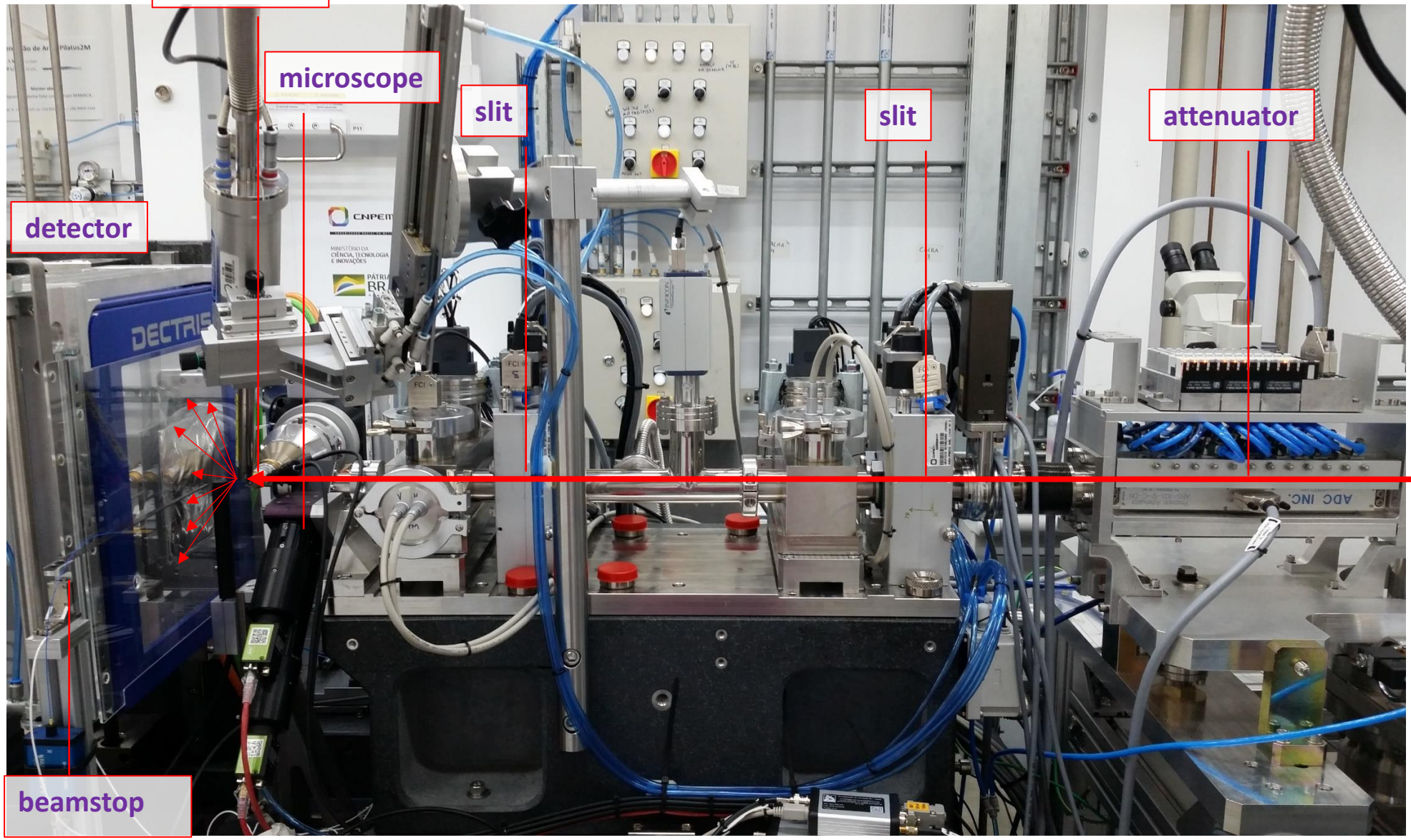
MANACA @ Sirius



MANACÁ @ Sirius



Experimental station

OAV microscope (Arinax)

Air bearing-based goniometer (in house)

PILATUS 2M detector (Dectris)

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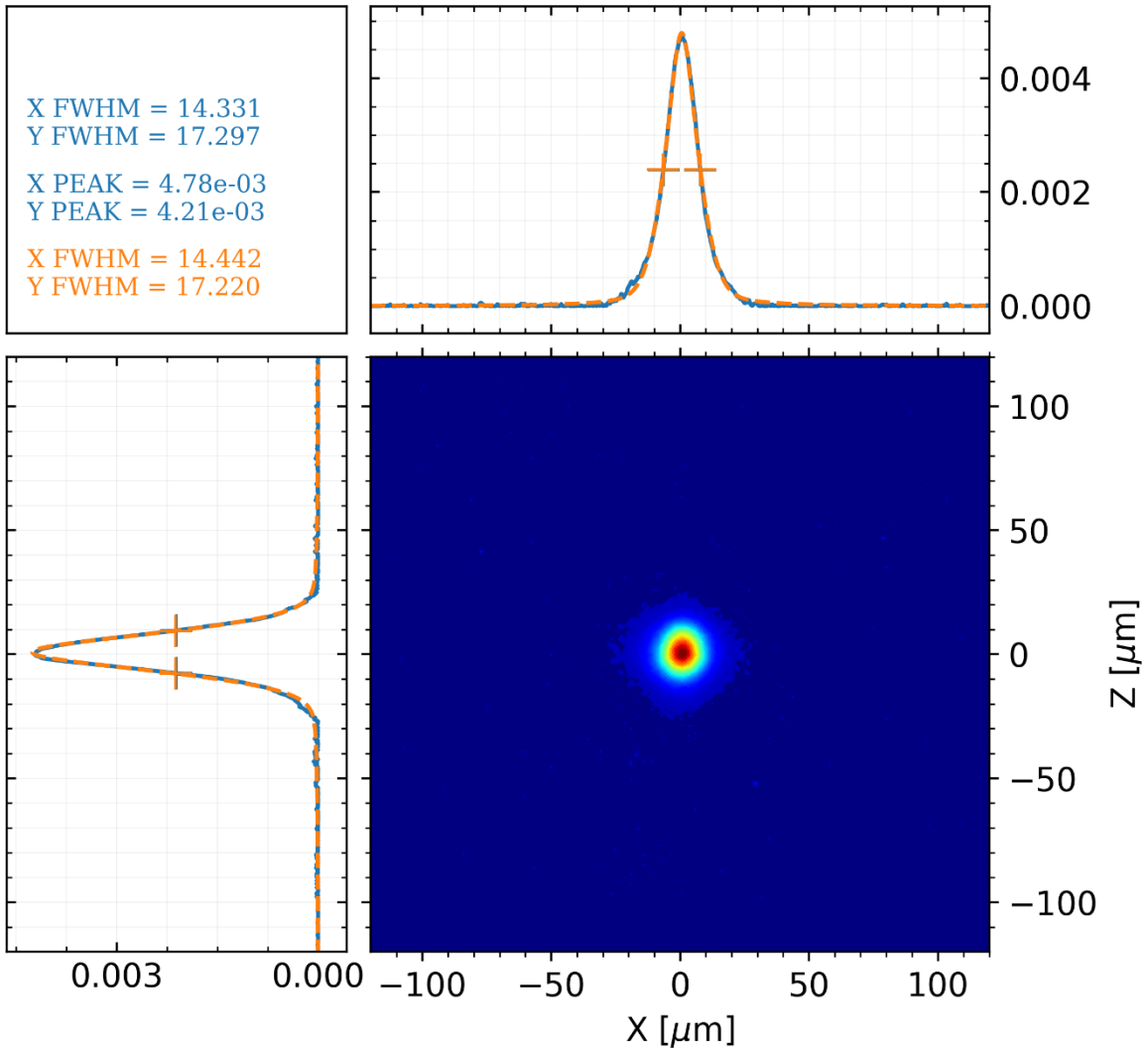
Beam size @ MANACA

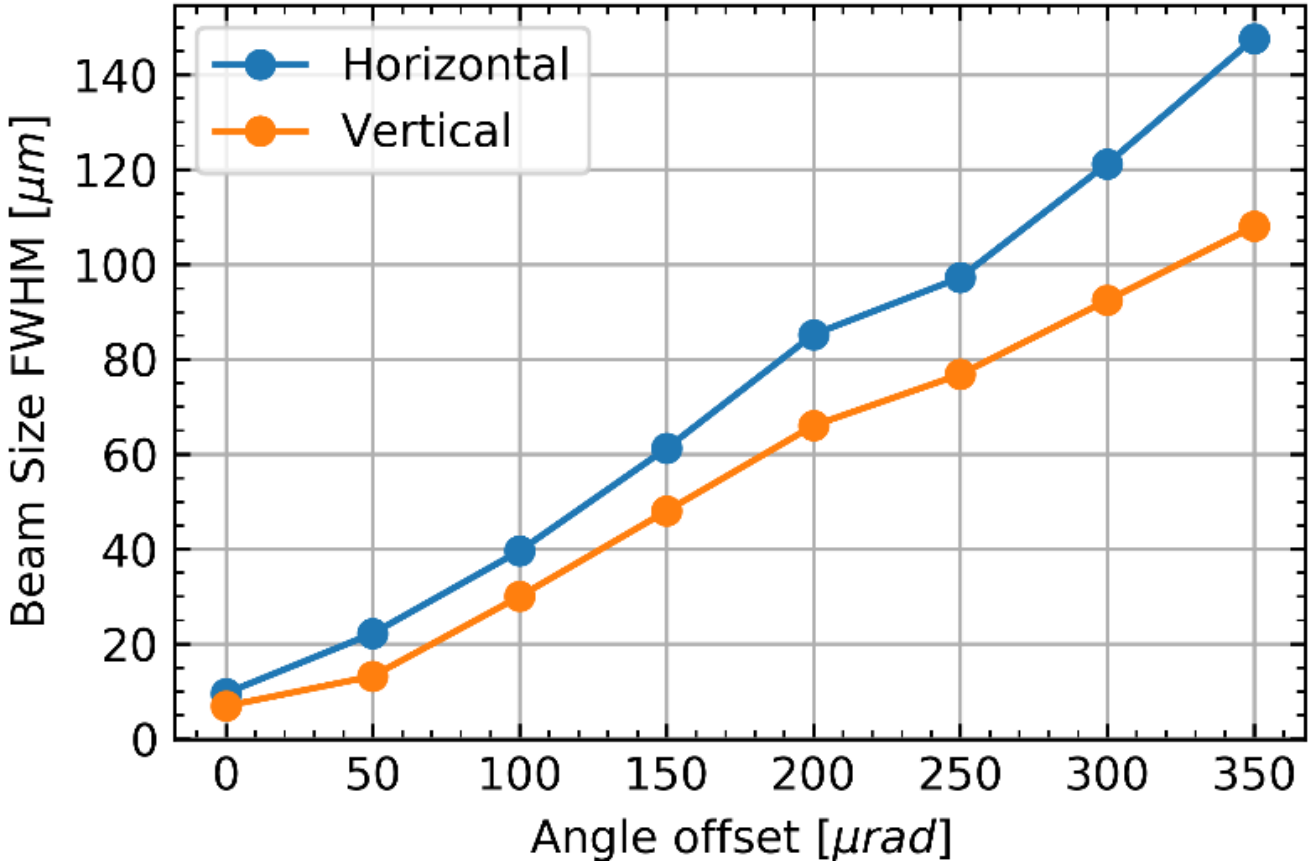
Small and adjustable beam size.

Nominal: 10 (H) x 7 (V) μm^2

Current operation: 20 (H) x 20 (V) μm^2

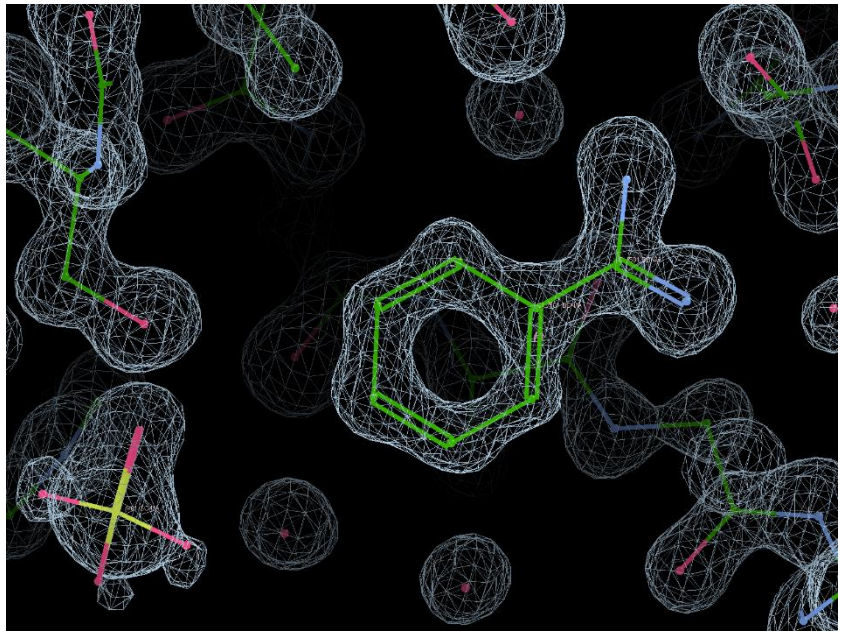
Flux: $6 \cdot 10^{11}$ ph/s/100 mA @ 12.7 keV





Energy range @ MANACA

The energy can be adjusted from 5.6 – 20 keV.

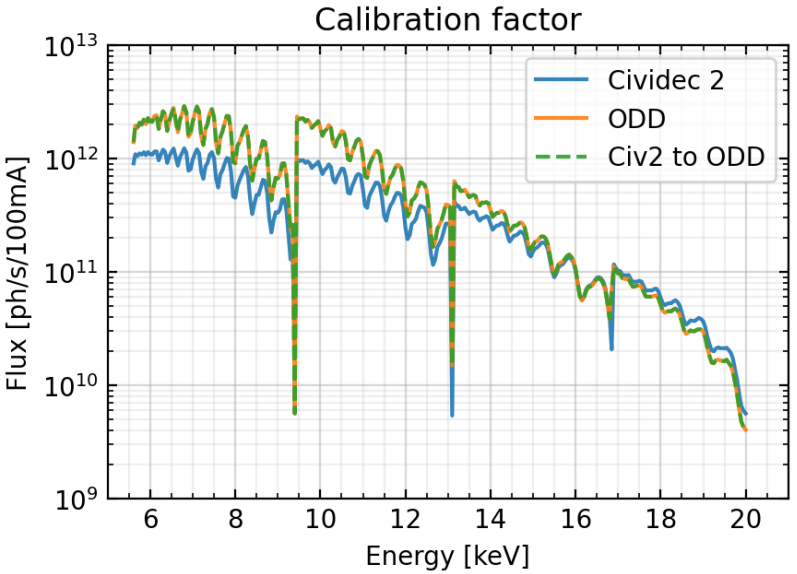


Benzamidine inhibitor bound to bovine trypsin (solved by native SAD).

Table 2. Data collection statistics of trypsin.

	Single crystal	Merged (4 crystals)
Energy (keV)	5.627	5.627
Resolution range	43.23 - 2.09 (2.165 - 2.09)	47.26 - 2.09 (2.165 - 2.09)
Space group	P 3_1 2 1	P 3_1 2 1
Unit cell (Å, °) (a=b c, α=β=90° γ=120°)	54.57 106.98	54.57 106.98
Total reflections	178059 (4294)	706448 (17246)
Unique reflections	11180 (867)	11405 (1078)
Multiplicity	15.9 (5.0)	61.9 (16.0)
Completeness (%)	97.81 (78.68)	99.78 (97.82)
Mean I/sigma(I)	33.76 (11.71)	54.10 (17.30)
Wilson B-factor	15.40	15.50
R-meas	0.0706 (0.0918)	0.0870 (0.1181)
CC1/2	0.999 (0.992)	1 (0.993)
Anomalous signal	1.307	1.859

Statistics for the highest-resolution shell are shown in parentheses.



High flux at low energy.

~2x10^{12} ph/s/100 mA @ 6-8 keV

Nascimento et al. Synchr. Rad. News, 34:5, 3-10 (2021).

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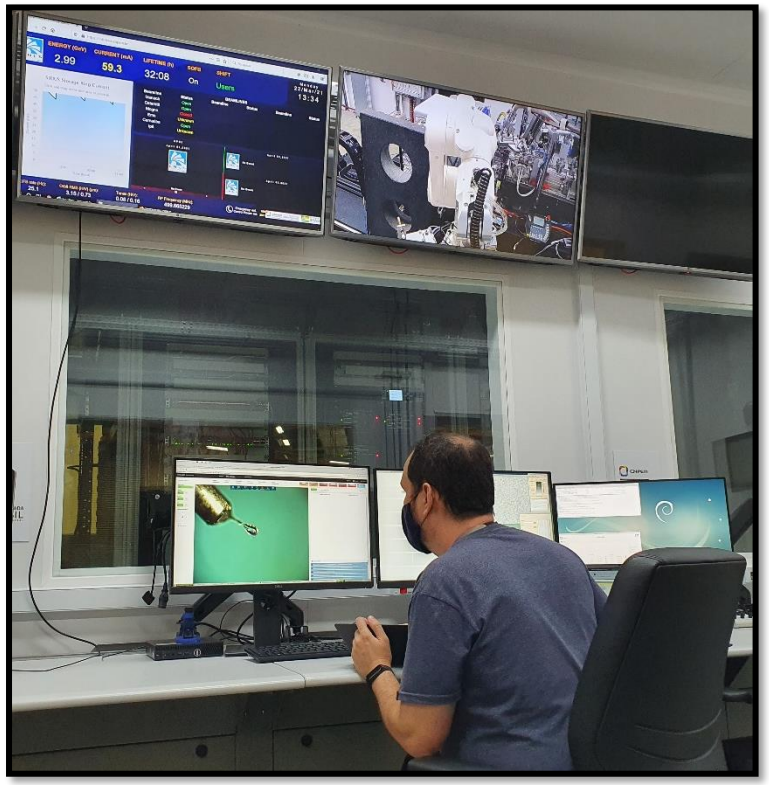
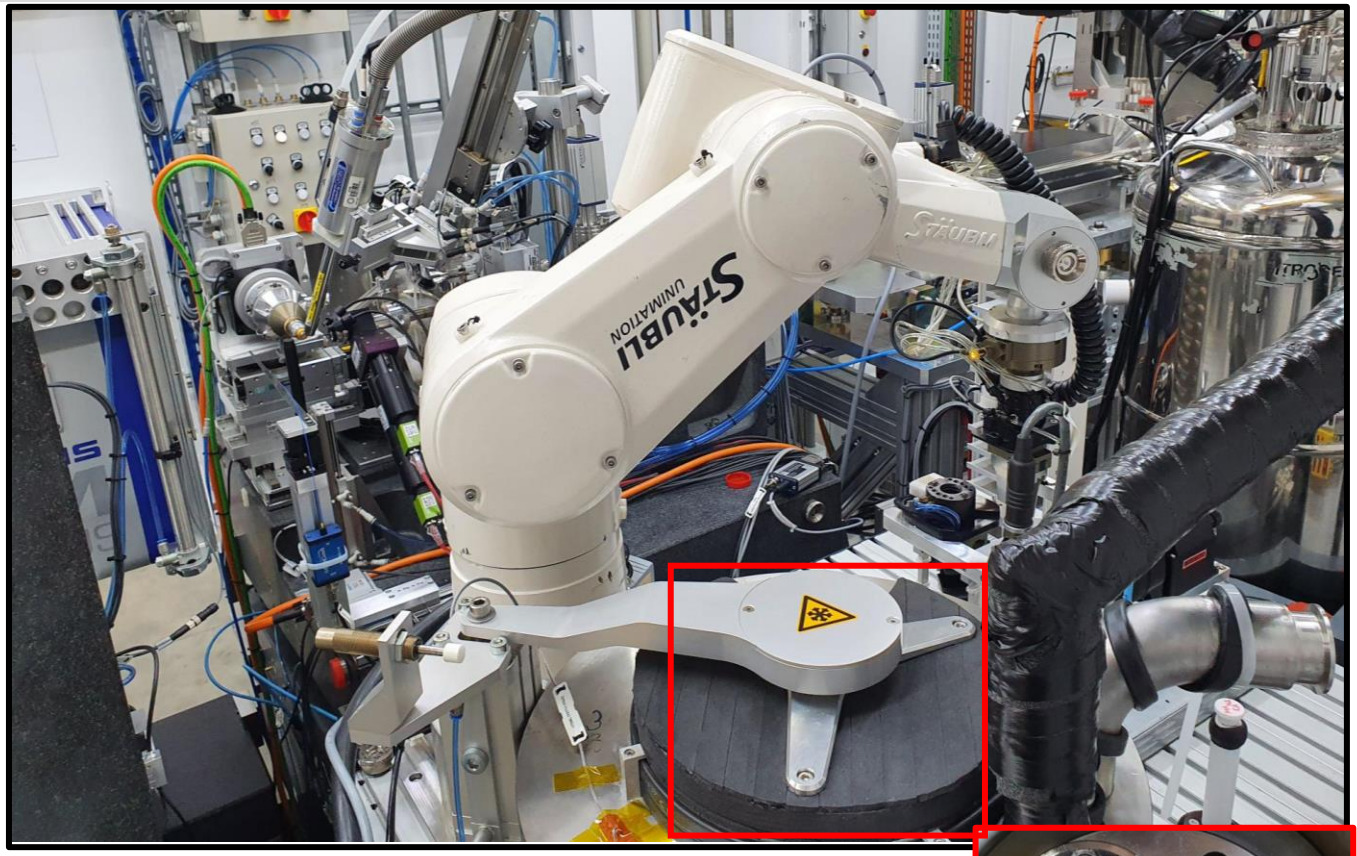
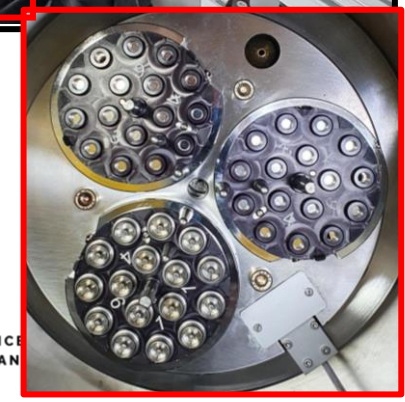
Sample Changer @ MANACA

Automatic and fast sample mounting.



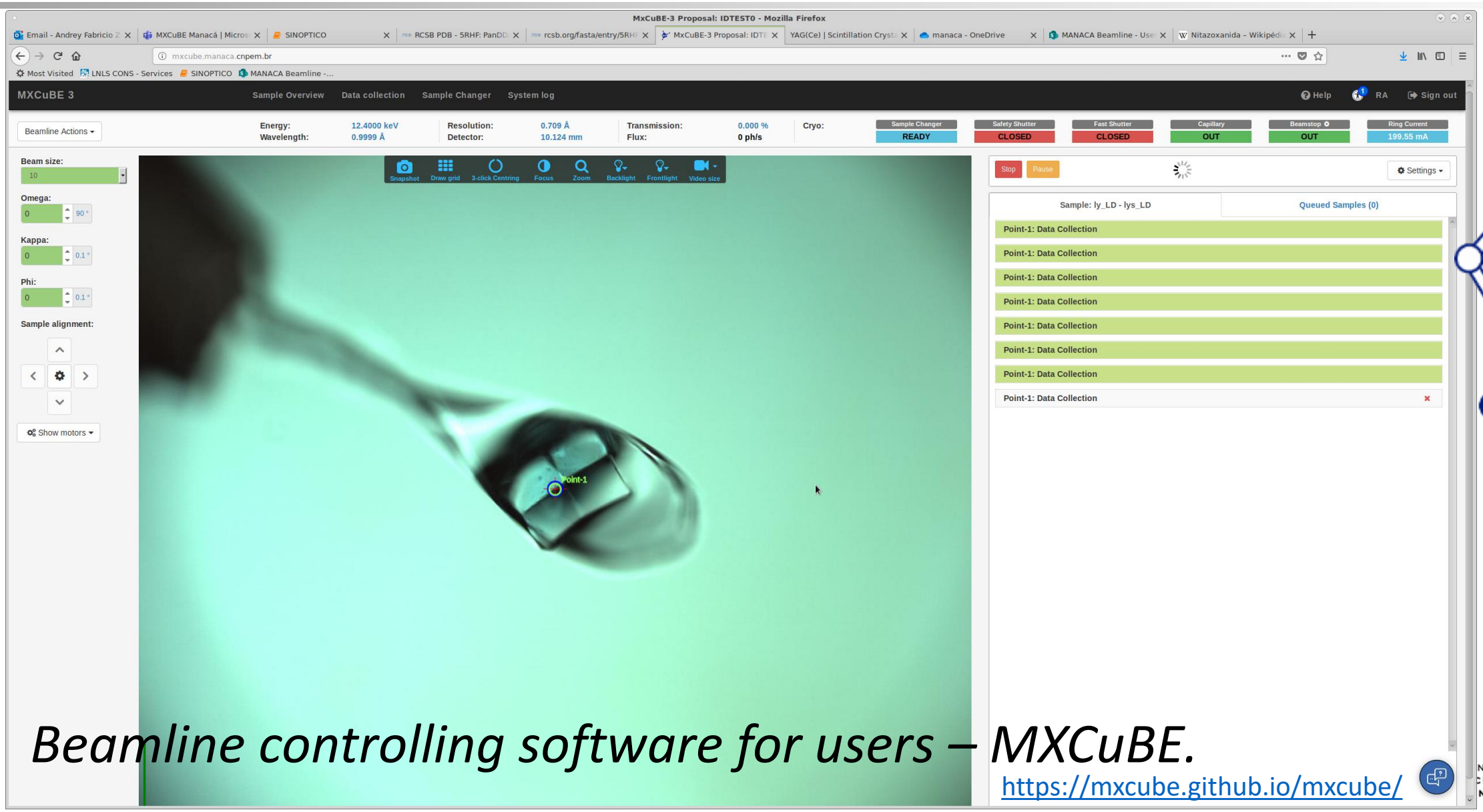




In operation with users since April/2021.

- Capacity for 48 samples (3 Unipucks; SPINE caps).
- Changes a sample in ~50 seconds (unmount/mount).
- Full user control through graphical interface (MXCuBE).

Friendly usage - MXCuBE @ MANACA



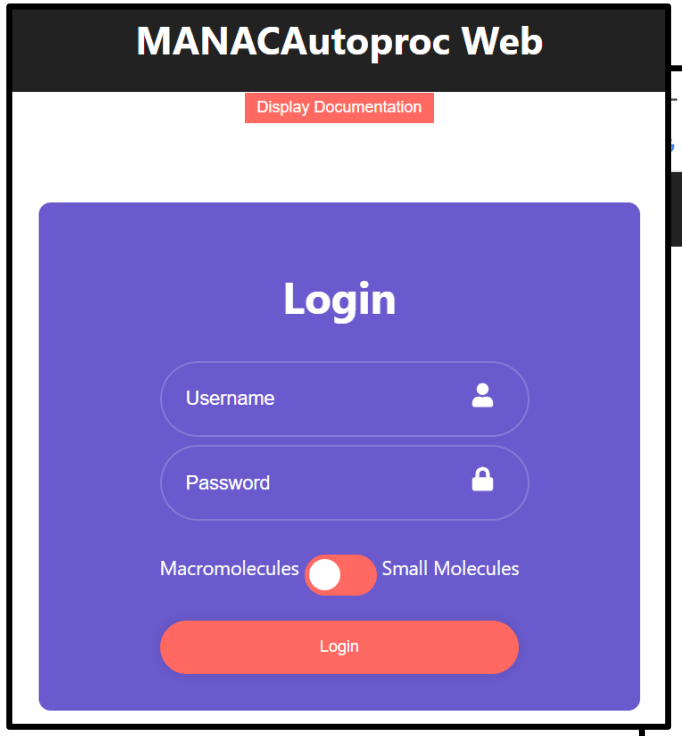
Beamline controlling software for users – MXCuBE.

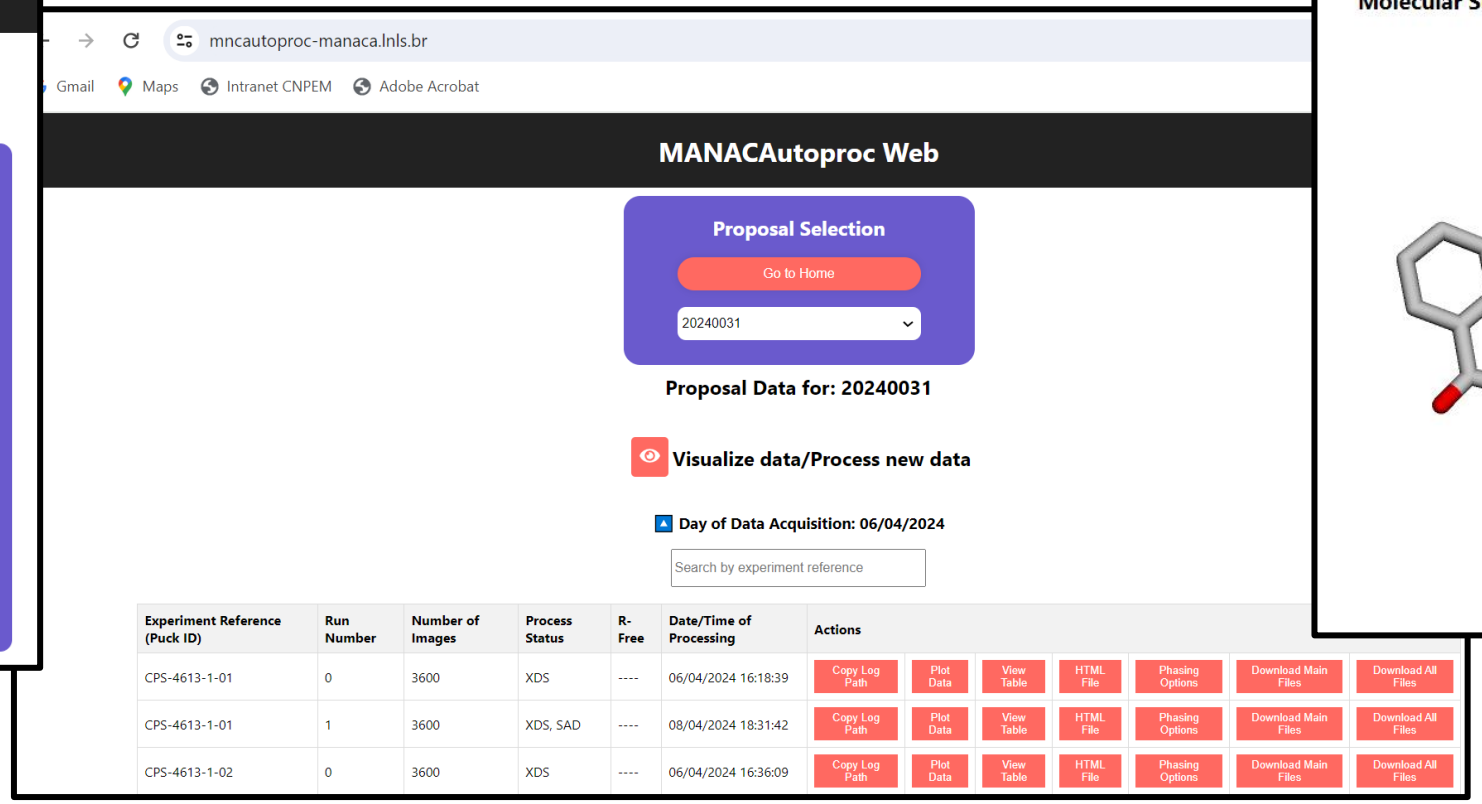
https://mxcube.github.io/mxcube/

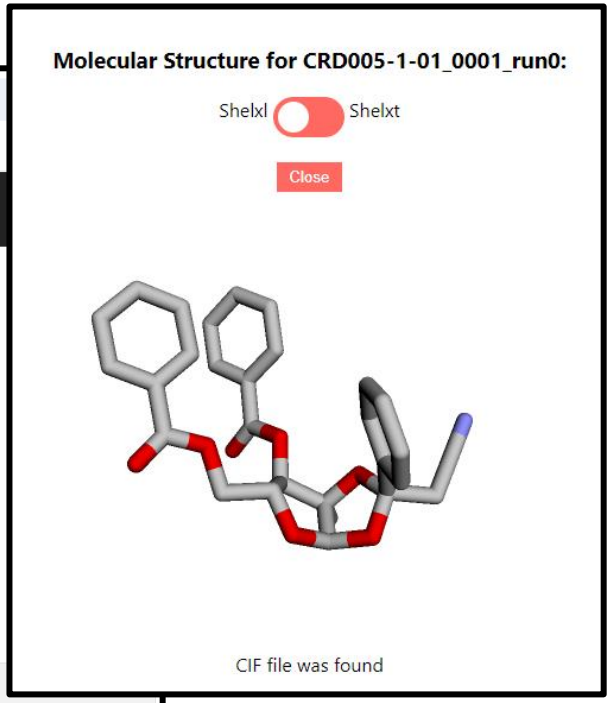
Data processing - MANACAutoProc

MANACAutoProc Web: interface for data processing.

No ssh connection or HPC setup required. Accessible from any OS through CNPEM's VPN.







For protein and small molecules.

https://mncautoproc-manaca.lnls.br

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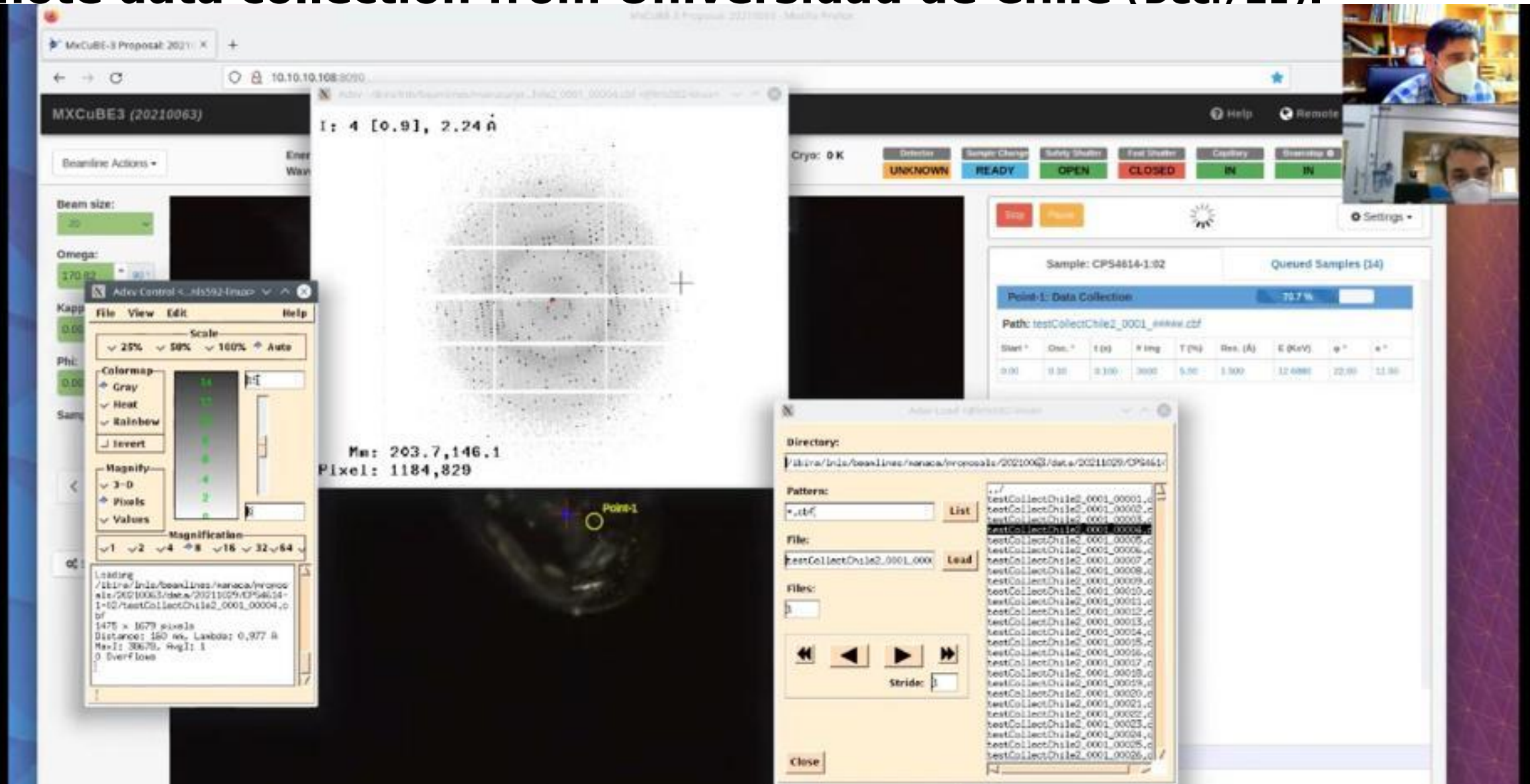
Remote Access @ MANACA

Remote data collection from Universidad de Chile (Dec./21).

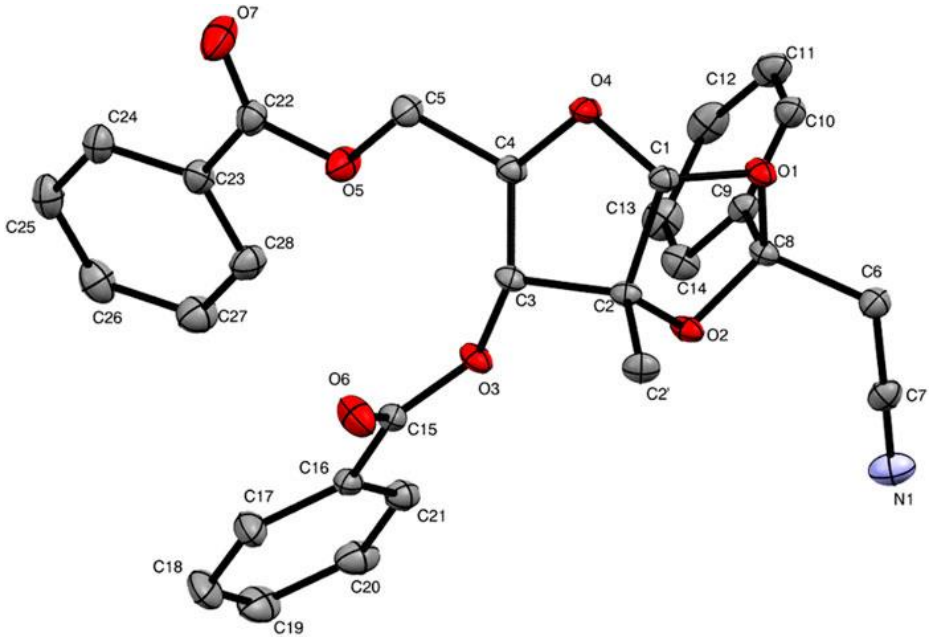


Recent developments

for protein and chemical crystallography

Chemical Crystallography – Small Molecules

a

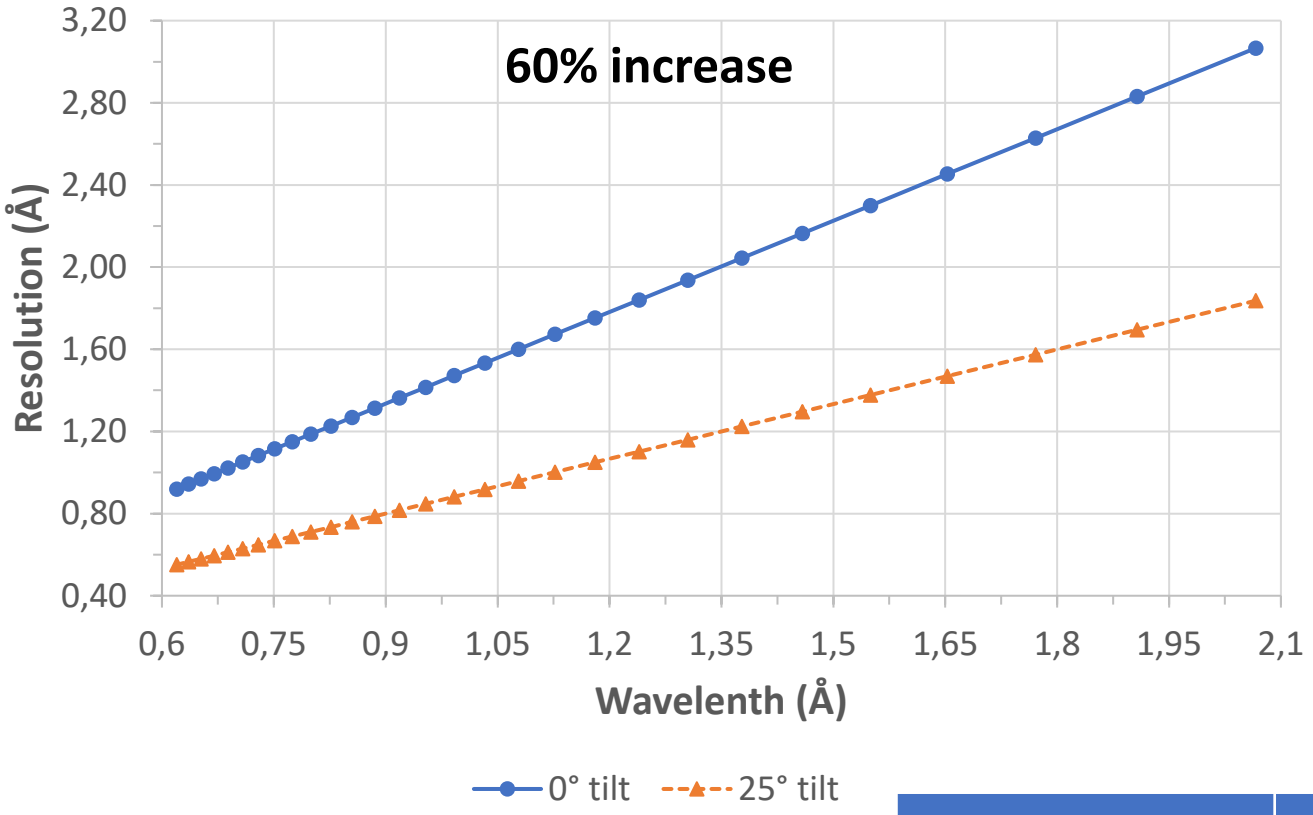


b

a, first structure of a small molecule solved at MANACÁ beamline (Naciuk et al., *Front. Chem*. 11, 2023). **b**, small molecule data collection by advanced users. After an one-week intensive work several high-quality data sets were collected, confirming the potential of MANACÁ beamline for Chemical crystallography Community. From left to right: Prof. Javier Ellena (USP, São Carlos), Prof. Leopoldo Suescun (Udelar, Montevideo), Dr. Andrey Nascimento (MANACÁ, LNLS), Prof. Alejandro Ayala (UFC, Fortaleza) and Prof. Florencia Di Salvo(UBA, Buenos Aires).

MANACÁ beamline – improvements and developments

Detector tilt: increase 2theta (*commiss.*).



Energy (keV) / Wavel. (Å)	Resolution (Å)	
	@112 mm	*tilted*
18.5 / 0.65	0.75	*0.60*
17.462 / 0.71 (Mo-K)	0.80	*0.63*
8.051 / 1.54 (Cu-K)	1.73	*1.37*

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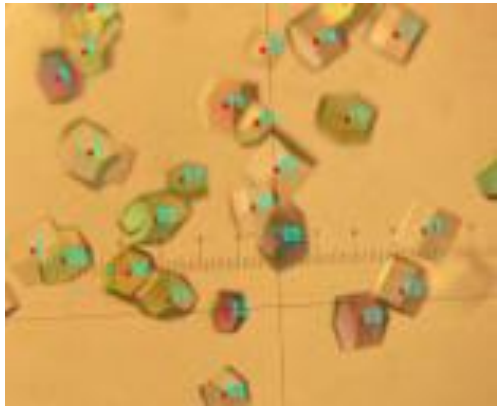
Room Temperature Macromolecular Crystallography

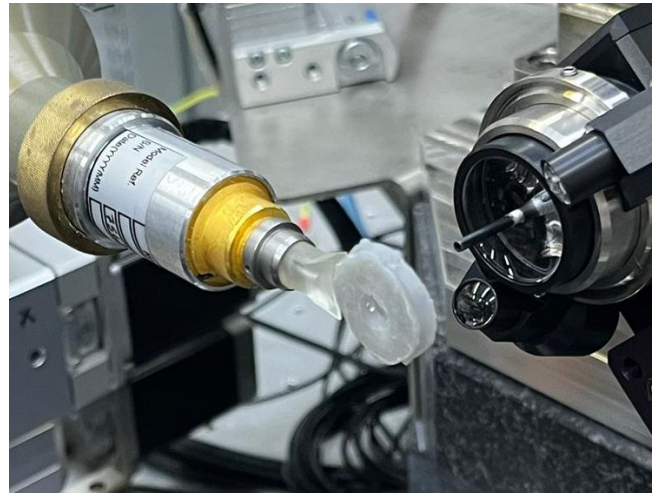
From plate to structure pipeline.

Crystallization

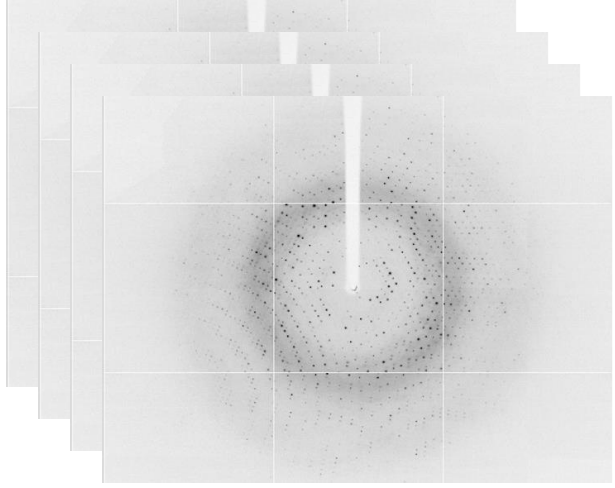
Mount



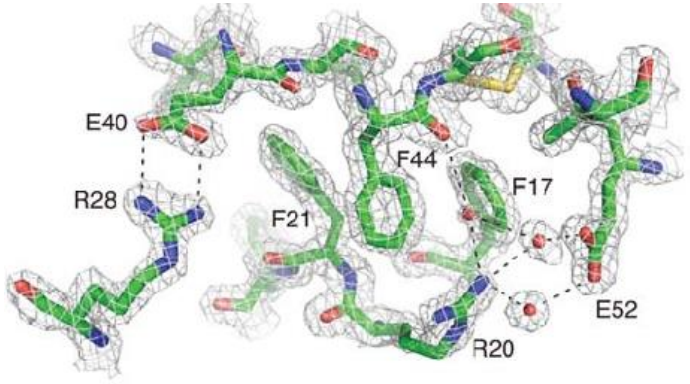
Crystal selection



Data collection
(small wedge, e.g. 10°)



Automatic multi-crystal data processing



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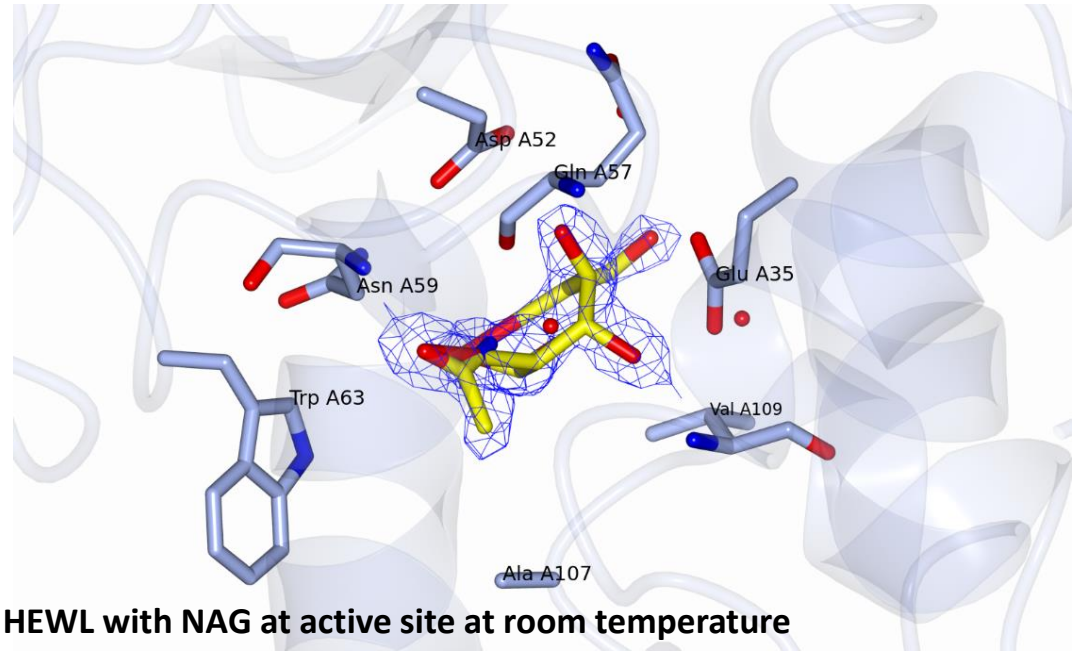
Room Temperature Macromolecular Crystallography

Automatic crystal selection (*deep learning*)

→ **Data collection**



HEWL with NAG at active site at room temperature

Automatic pipeline!

Phasing & Refinement

phaser, phenix.refine

White et al., J. Appl. Cryst. **45** (2012).
Gildea et al. Acta Cryst. D **78** (2022).
Kabsch et al. Acta Cryst. D **66** (2010).

Data reduction options

mncautoproc_multixtal

wedge 1 | wedge 2 | wedge *n*

xds

data set 1 | data set 2 | data set *n*

clustering
blend, ccCluster, xscale_isocluster

xscale

merged .HKL (*all*)

xdscc12

Data set filtered list (ΔCC1/2 cutoff)

xscale

final merged .HKL (*"best data sets"*)

xia2.multiplex

wedge 1	wedge 2	wedge *n*
dials.import	dials.import	dials.import
dials.find_spots	dials.find_spots	dials.find_spots
dials.index	dials.index	dials.index
dials.refine	dials.refine	dials.refine
dials.integrate	dials.integrate	dials.integrate

→ xia2.multiplex

CrystFEL script

image 1 | image 2 | image *n*

Geo. file generation Threshold selection.

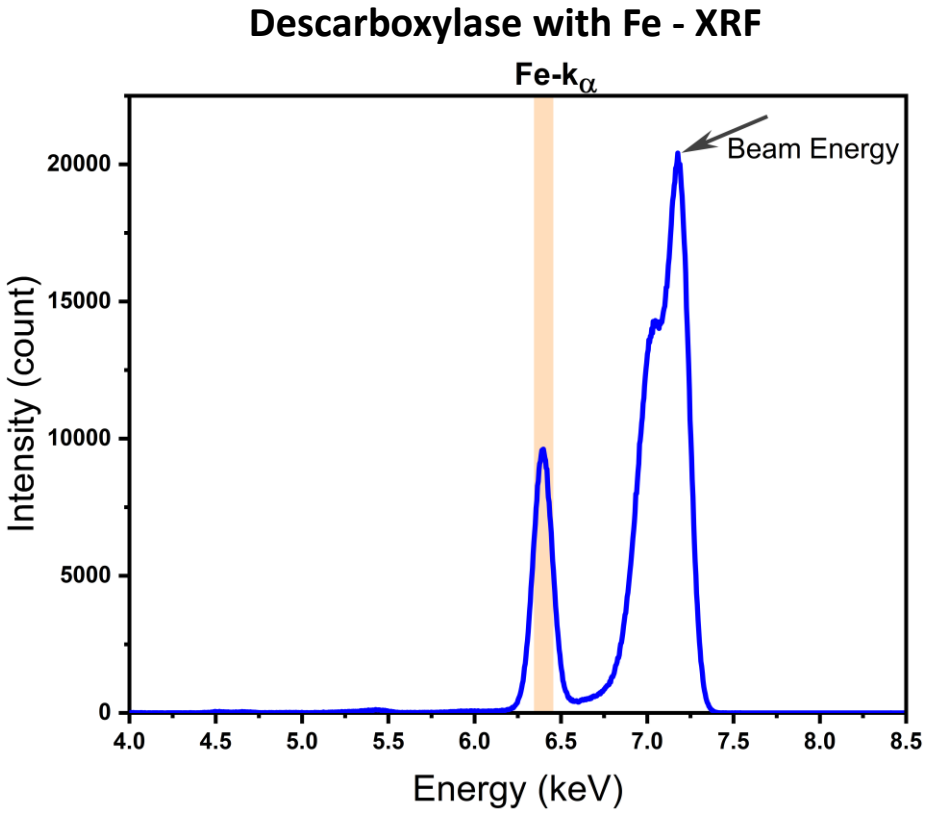
CrystFEL

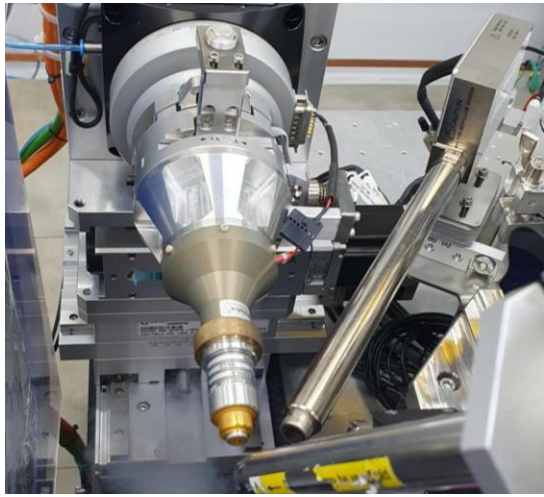
→ **.mtz file**

X ray Fluorescence @ MANACÁ

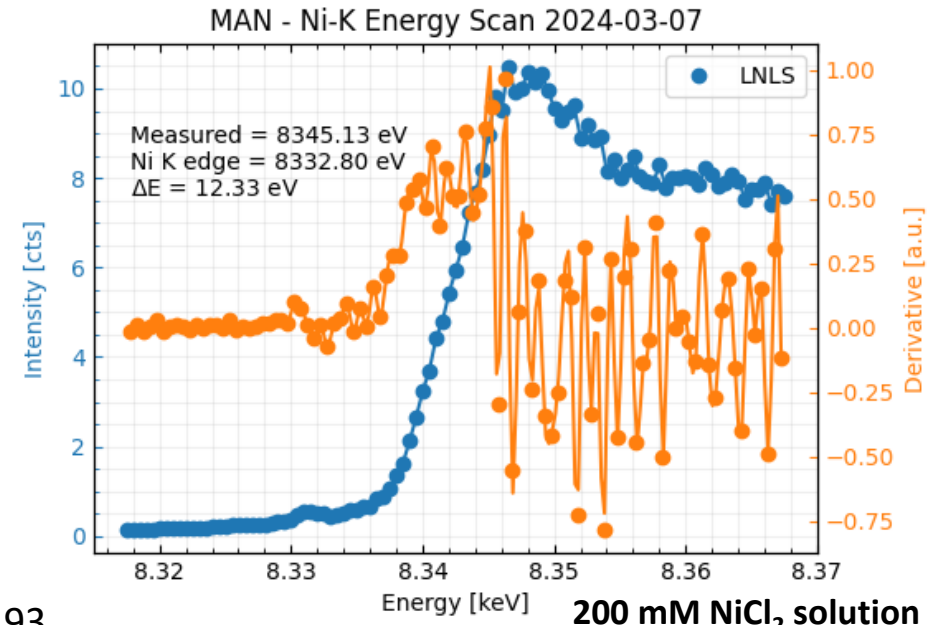
MAD scan and XRF (*under commissioning*).

Phasing and element search in the crystal.





MANACÁ's first fluorescence scan

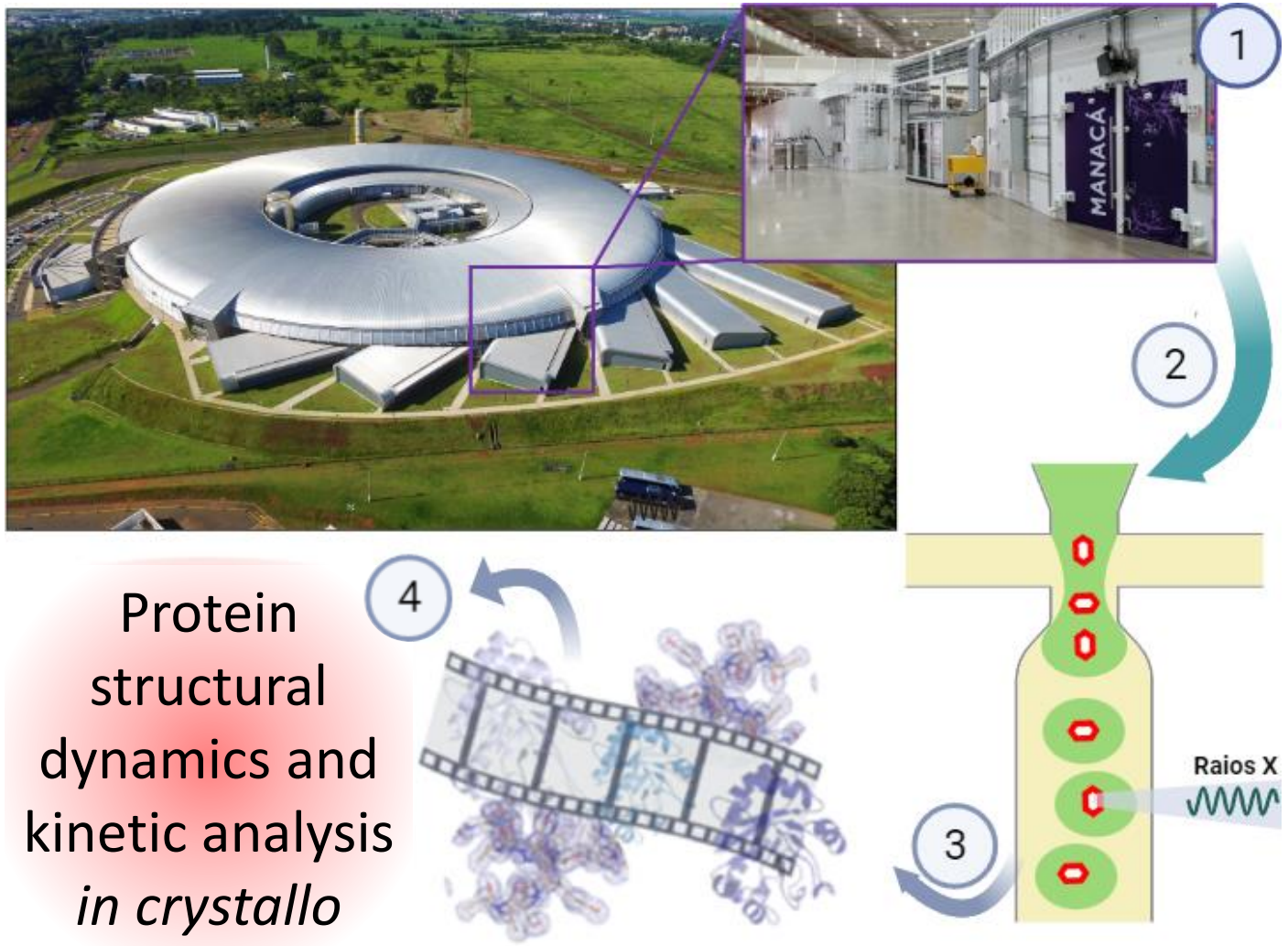


200 mM $NiCl_2$ solution

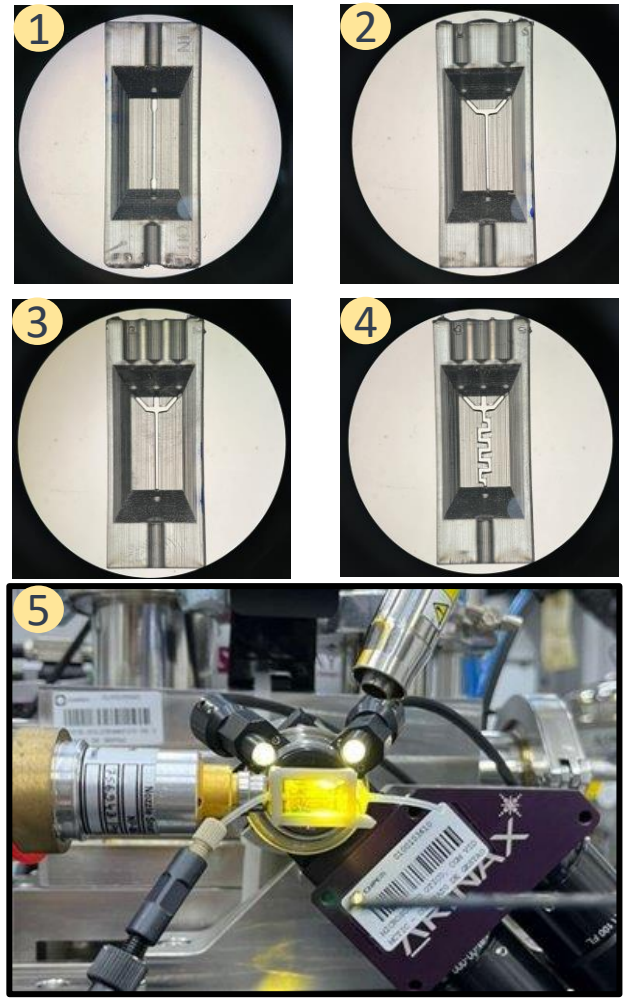
Data from Dr. Renan Miyamoto and Dr. Leticia Zanphorlin, LNBR/CNPEM. Proposal #20241193

Time-resolved and serial crystallography

Time resolved crystallography for study reaction mechanism of enzymes at atomic level.



Protein structural dynamics and kinetic analysis *in crystallo*



Microfluidic sample holders for serial crystallography. ***Under development.***

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MXCuBE at LNLS/Sirius

Status Report

on behalf of Nicolas Moliterno (COMP)

MXCuBE status

- Production Version:
  Web MXCuBE3 - Backend and Frontend

- Development Version:
  Web MXCuBE3, Web MXCuBE4, mxcubecore

MXCuBE status

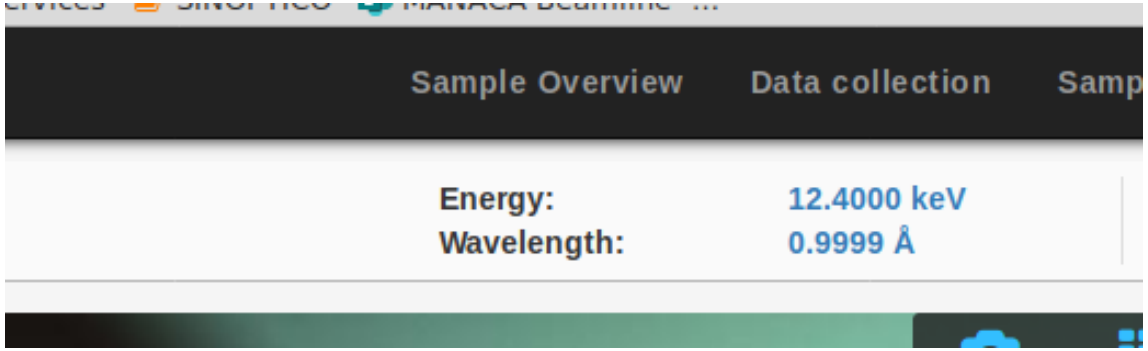
- Other Deployments
  - Exploring Bluesky to replace legacy scans relying on older Python versions

- Cybersecurity
  - MXCuBE is accessed via VPN
  - Cybersecurity is managed by the CNPEM-IT Team.

Developments since last meeting

- Refactor: EPICSActuator Class
  - Integrated new devices.

- Update: LNLSEnergy Class
  - Previously, only allowed reading DCM Energy. Now it can set Energy as well.
  - IOC DCM refactor enabled new functionalities related to energy, overcoming previous equipment configuration limitations.



Developments since last meeting

- BlueSky with MXCuBE in LNLS
  - New class LNLSWorflow
  - Include WF mesh scan
  - Live view of grid design
  - Integrate a bluesky API for saving metadata
  - Scan control and live monitoring of generated data
  - Helical scan in development

Developments since last meeting

- Refactor: LNLSTransmission Class & Associated Equipment
  - Previously: A Python script controlled transmission actuators, performing calculations using variables like energy.
  - Now: The script is replaced by an EPICS IOC featuring:
    - State Machine with an ALU (Arithmetic Logic Unit).
    - This update replaces Python-script-controlled equipment within MXCuBE, leaving only scans, post-processing scripts, or motor subroutines.

- Update: LNLSDetDistMotor Class
  - With the granite base update for the detector, movements are now handled by kinematics, replacing the real motor.

Plans for the next six months

- Update scan routines
- Integrate new equipment (e.g. mini-kappa, det. tilt), update of LNLS class files
- Update LNLSCollect
- Test MXCuBE 4/5 with new scan routines (bluesky).

Beamline plans for near future

- Integrate RT data collection.
- Implement a web diffraction image viewer (Braggy, h5web, ?).
- Improve automatic data processing at MXCuBE (currently only data reduction is performed).
- Implement characterization software (EDNA/DOZR).
- Start the implementation of a LIMS (ISPyB, ICAT, ?).

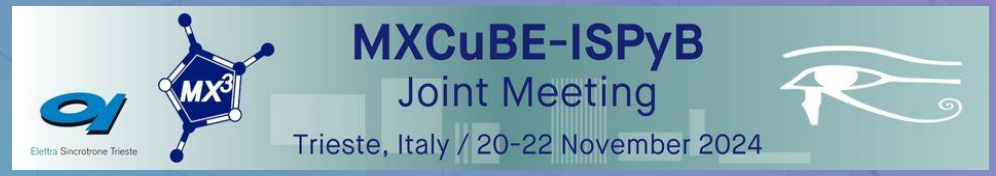
THANK YOU!

GRAZIE!

OBRIGADO!

Andrey Nascimento
andrey.nascimento@lnls.br

https://lnls.cnpem.br/facilities/manaca-en/



CNPq

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