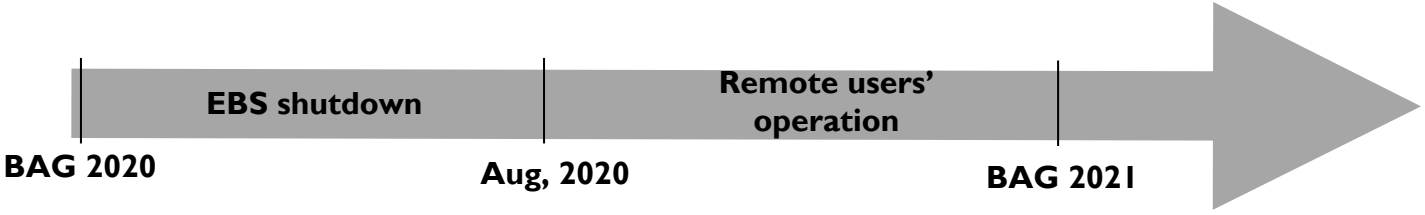
Updates from Serial Synchrotron Crystallography BAG

Shibom Basu
Staff Scientist

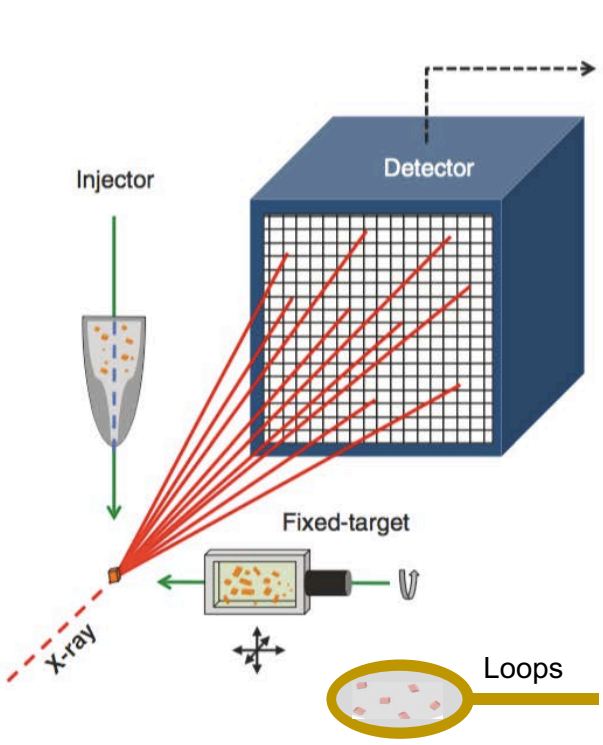


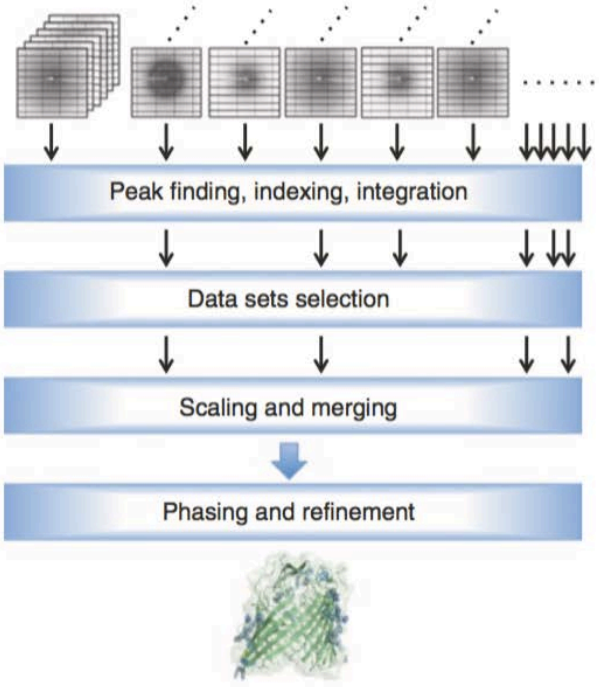
A light for science ESRF

EMBL

Serial Synchrotron Crystallography (SSX) – what can be done?

- SSX
  - ≤ 20 µm
  - Crystals can be in loop/mesh/chip or jet
  - 100 K or RT
  - Small wedges or stills





Diederichs and Wang, ***MiMB***, 2017

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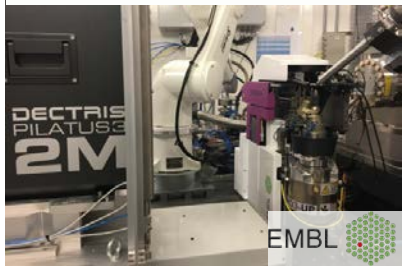
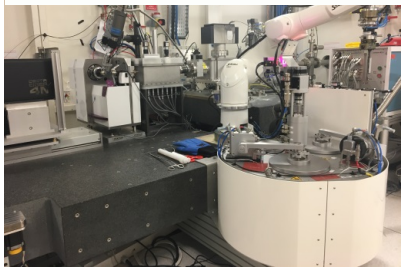
Serial crystallography at ESRF-EMBL – after upgrade

ID23-2 (μ focus)



- Fixed energy at 14.3 keV
- μ focus beam (5 x 5 μm^2)
- SSX experiments with injectors, solid supports – including *Crystal Direct* plate + loops
- SSX with heavy element SAD
- Cryo as well as RT data collection
- Pilatus 2M detector

ID30-A3 (μ focus)



- Fixed energy at 12.81 keV
- Fixed beam size 15 x 15 μm^2
- SSX experiments with injectors, solid supports
- Cryo as well as RT data collection
- EIGER 4M detector

Old ID29 – EBLS8

- Whole new end-station for dedicated SSX experiments
- Time-resolved SSX experiments

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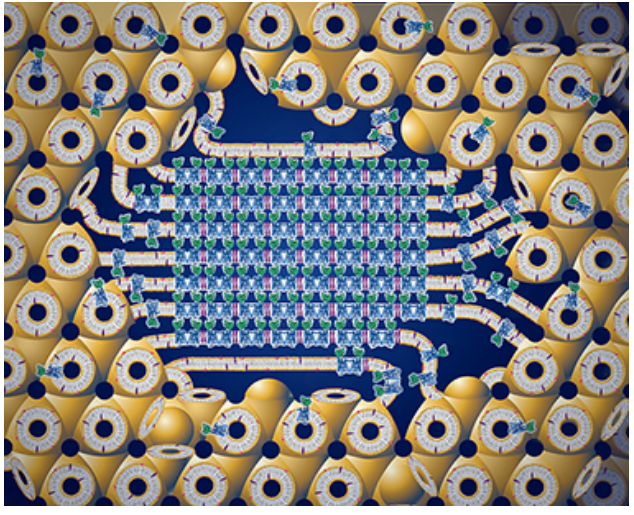
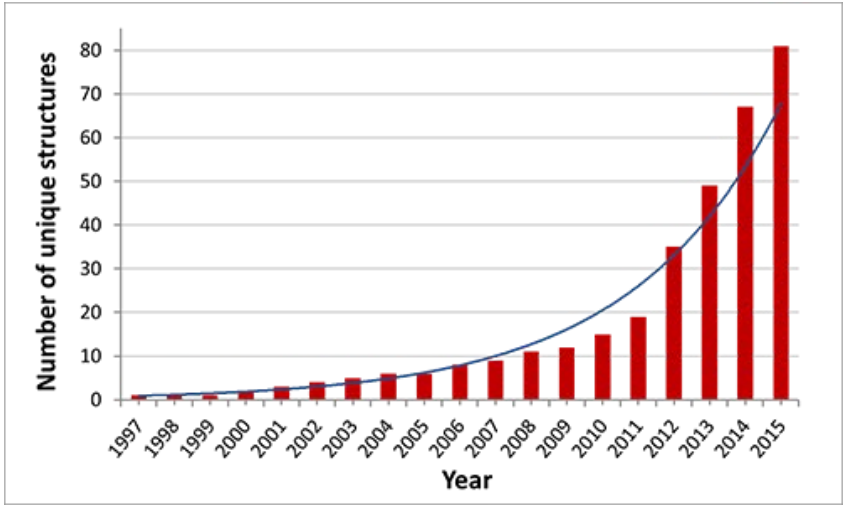
LCP crystallization is challenging.. Only a handful structures



Total structures 257; Unique structures 81



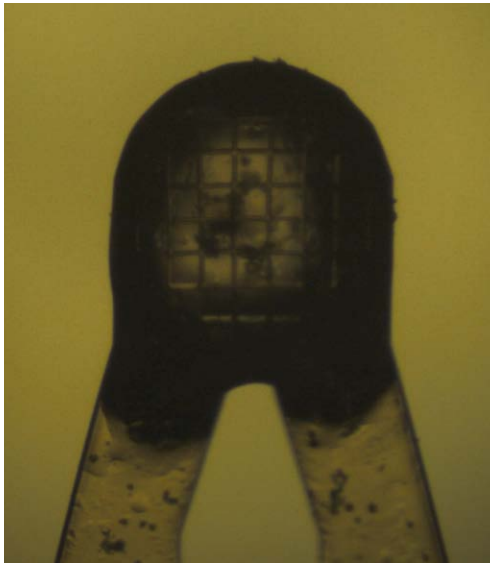
Lipidic Cubic Phase (LCP) crystallization

- Facilitated the study of many membrane proteins
- Difficult to manipulate crystals in LCP media
- Produce micro-crystals, entailing SSX/SFX method

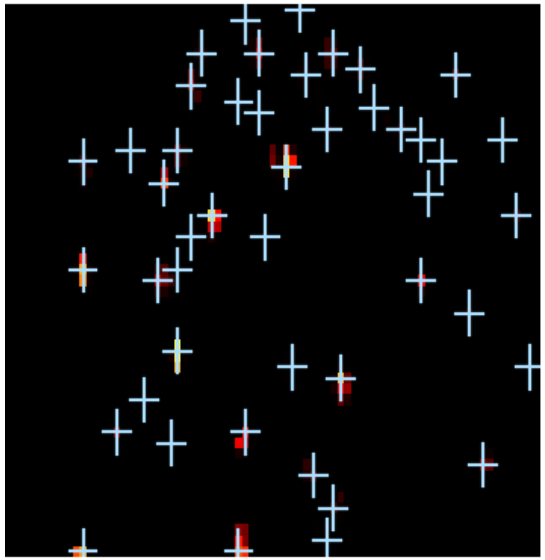
https://cherezov.usc.edu/tools_gsp.html

EMBL

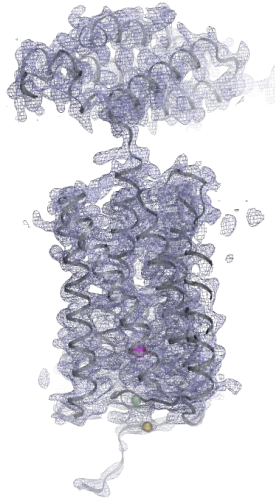
SSX measurment on an integral human membrane protein – ID23-2



LCP bolus with microcrystals on mesh loop



Heat map with identified crystals locations on the mesh-grid

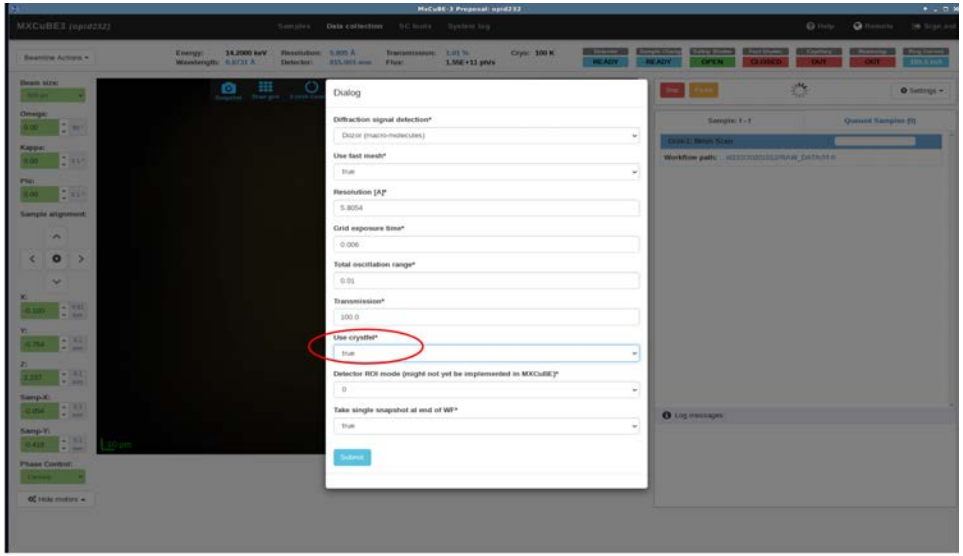


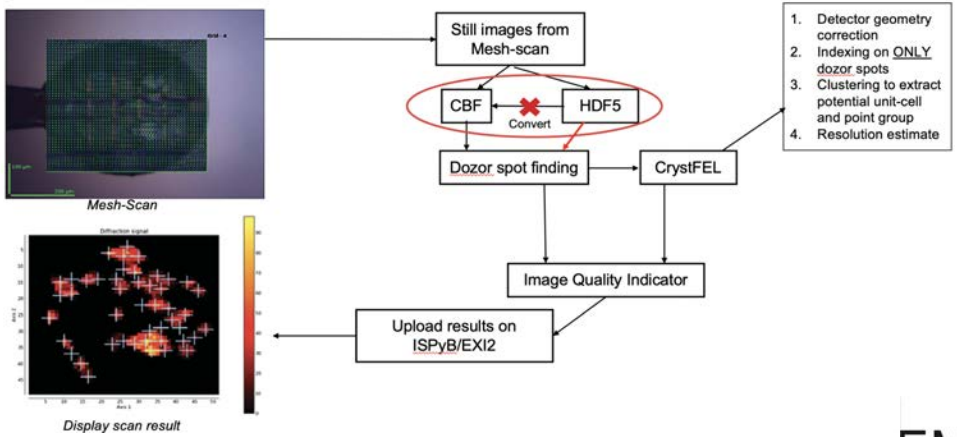
2.5 Å resolution
Map at 1.0 σ

Granier Lab

EMBL The European Synchrotron | ESRF

New development for Mesh&Collect





- CrystFEL software has been integrated in Mesh scan workflows and can be triggered from MxCUBE3
- Aim is to support Injector or solid-support based SSX experiments
- Part of new EDNA2 written by Olof Svensson

Automated Crystallography Pipelines

Marquez Team

EMBL

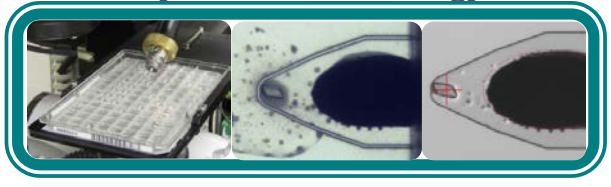
iNEXT → iNEXT Discovery

123 Scientists 14 Countries

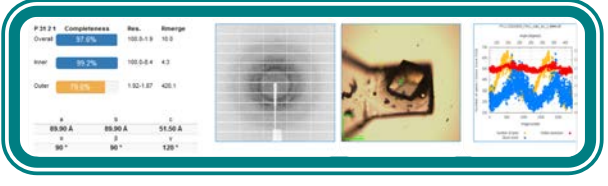
Starting Febr. 2020

High-throughput crystallization

CrystalDirect™ Technology



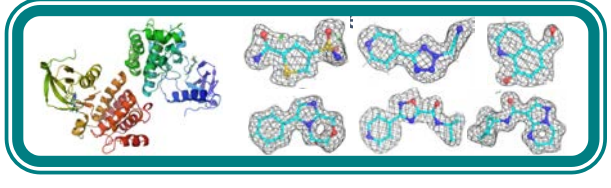
Synchrotron beamlines



ESRF Petra3

SLS

Automated data processing



Online Crystallography

Fully automated, protein-to-structure Pipeline

Compound Screening

Focused libraries

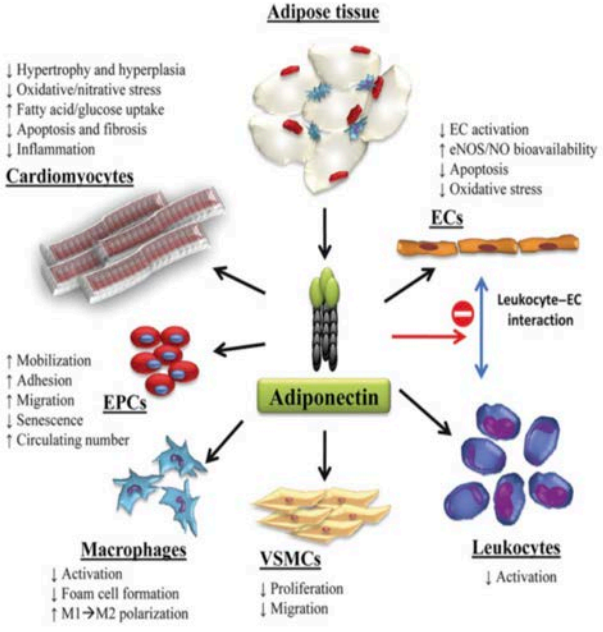
Fragment Library Screening

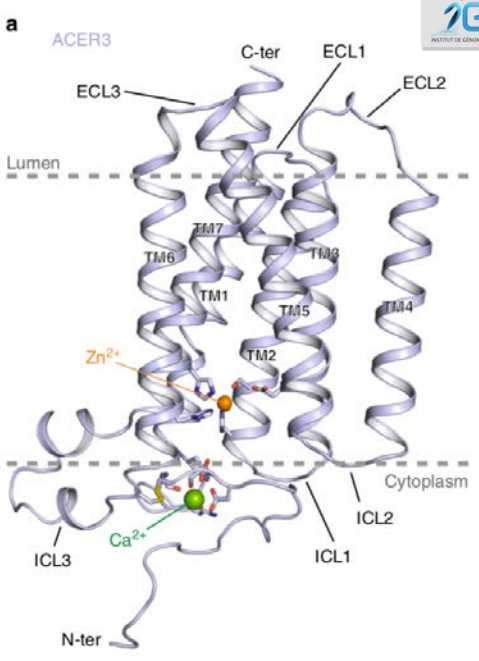
Large scale, fully automated fragment screening

EMBL ESRF ibs PSB EMBL isbg

Adiponectin receptor 2 (ADIPOR2) and Alkaline ceramidase (ACER3)

- Seven transmembrane enzymes
- Pathophysiological importance
- Important drug target
- Lipid cubic phase crystallization
- Molecular Wt. ~45 kDa

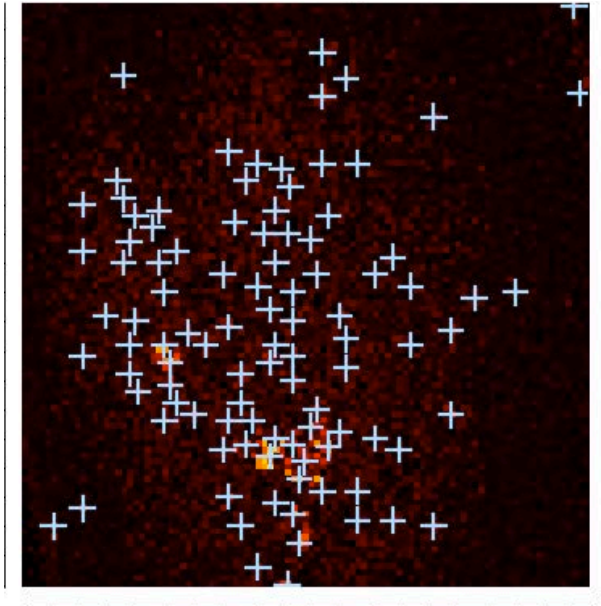




Vasiliauskaité-Brooks, Healey et al., *Nat. Comm.*, **2018**

ADIPOR2 SSX – ID23-2

LCP bolus with microcrystals on CrystalDirect loop



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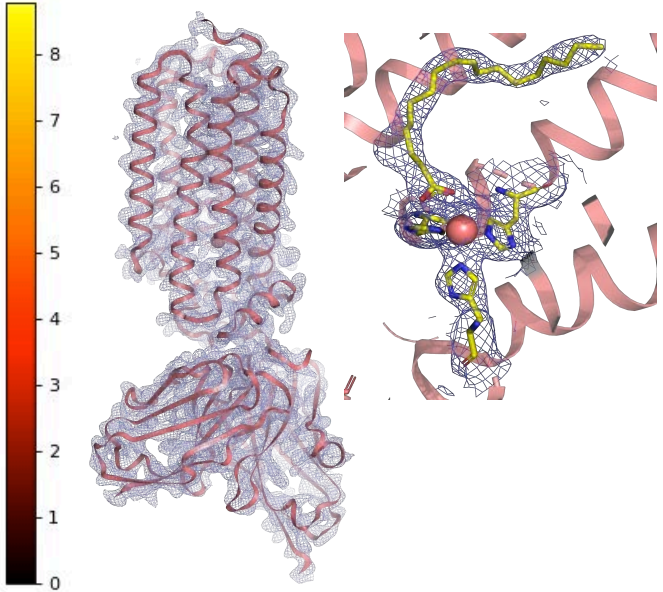


2.4 Å resolution
Map at 1.0 σ

Robert Healey, IGF
Florine Dupeux, EMBL
Anne Sophie-Humm, EMBL
Jose A Marquez, EMBL

EMBL

The European Synchrotron | ESRF

Summary and Outlook

LCP crystallization in CD plates
- High-throughput
- automated

Cryo-cooling harvesting and data collection
- Full automatic crystal harvesting in LCP and sponge phase
- Isolated crystal or full drop harvesting
- Full data set with only few loops

Room temperature *in situ* data collection
- Easy set-up at most MX beamlines
- Very low sample consumption

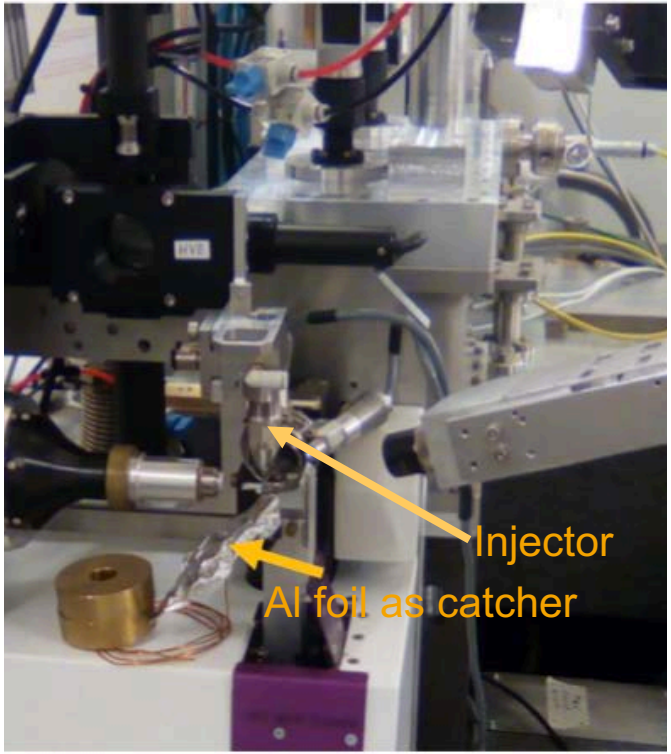
Enable to do soaking experiments
- sets premise for SSX based ligand screening in future

Automated data processing with in-house tools

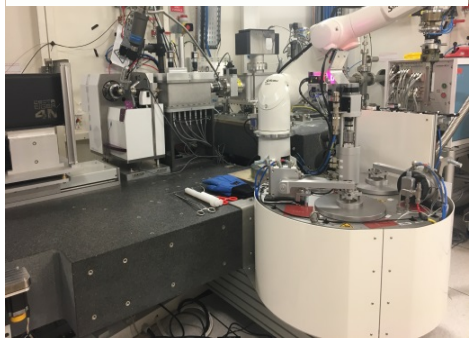
- 1.5 TB diffraction data from a membrane protein, co-crystallized with ligand collected
- 10 TB diffraction data for SSX based Fragment screening campaign on ADIPOR2 membrane protein collected
- Data processing is continuing

Contacts:
- Shibom Basu
- Jose A. Marquez

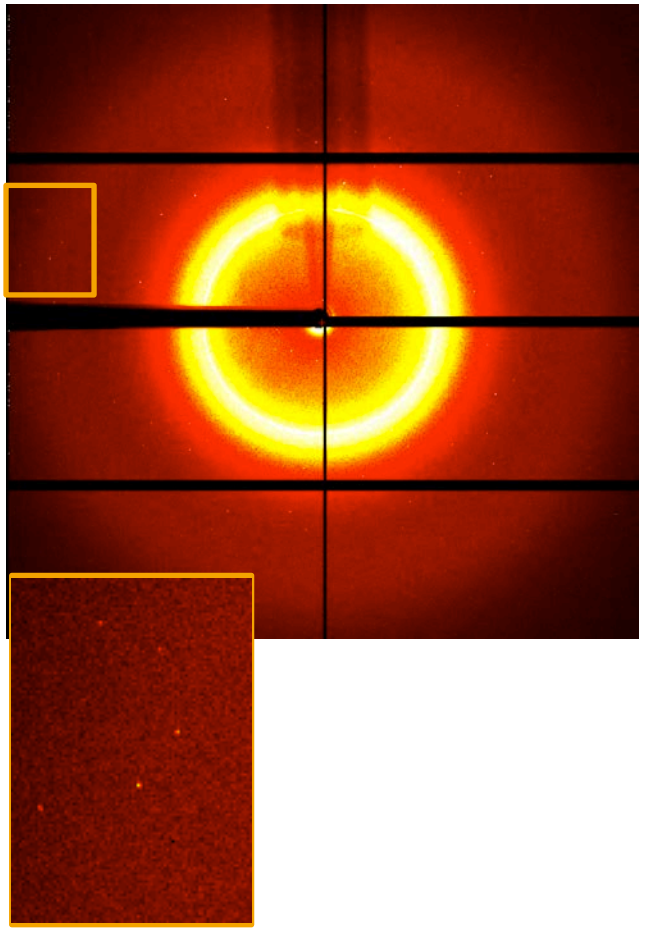
SSX with a visocus injector at Massif-3 beamline



ID30-A3 (μ focus)

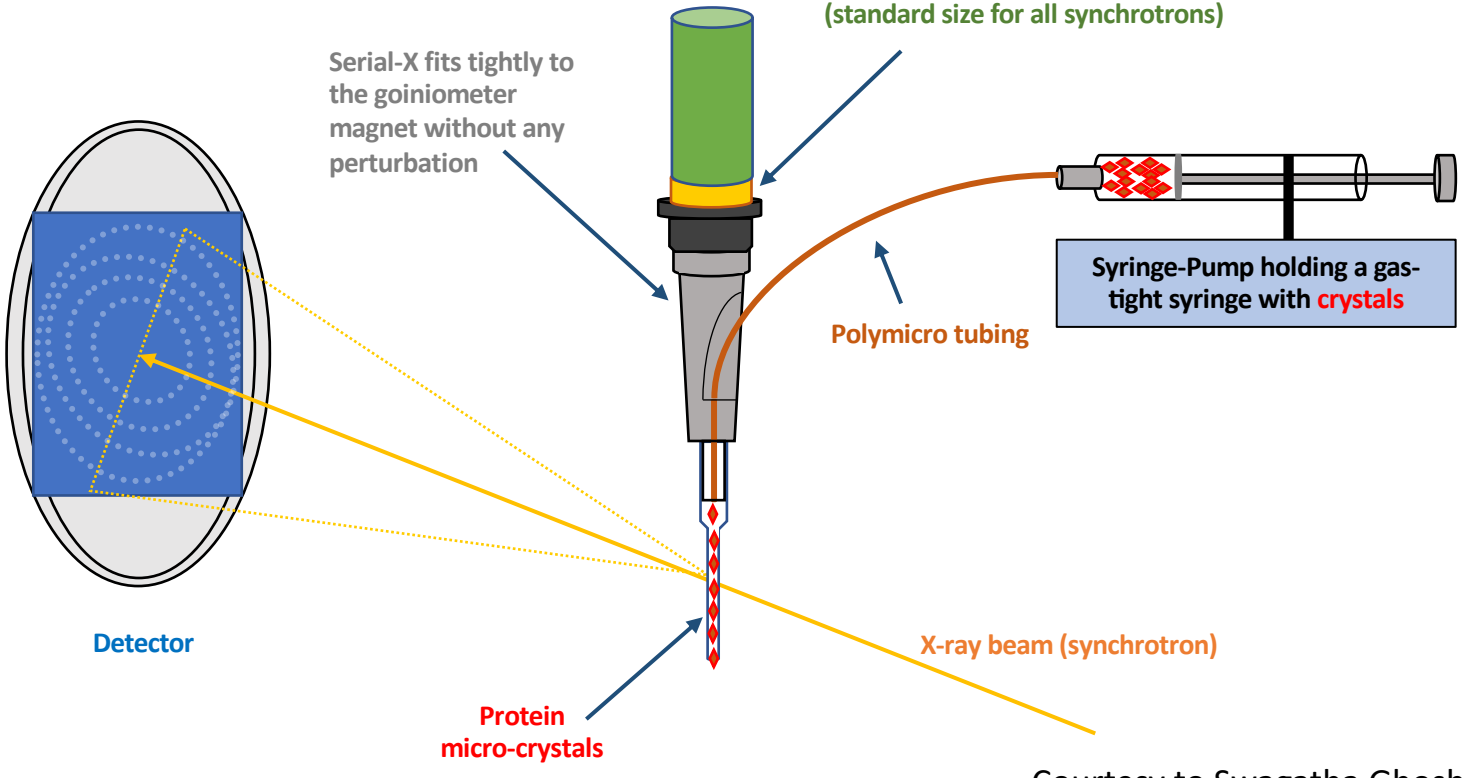


- Fixed energy at 12.81 keV
- Fixed beam size 15 x 15 μm^2
- EIGER 4M detector
- Lysozyme with SuperLube



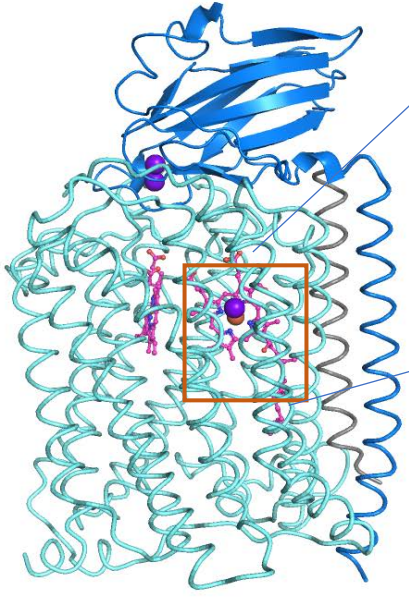
Daniele D Sanctis, ESRF
Gianluca Santoni, ESRF

Serial-X – a capillary based sample delivery tools for SSX

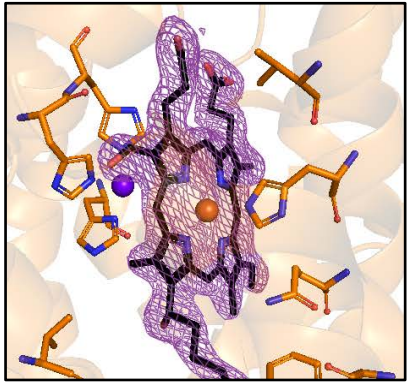


Courtesy to Swagatha Ghosh
Richard Neutze's Group

SX structures of *ba3-type* cytochrome c oxidase using different methods and X-ray sources

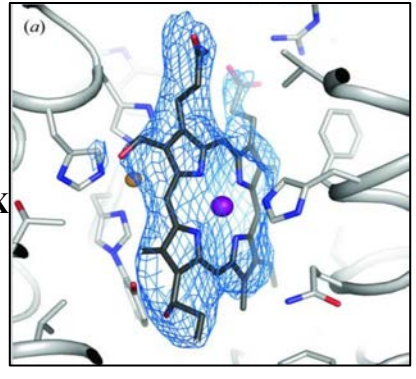


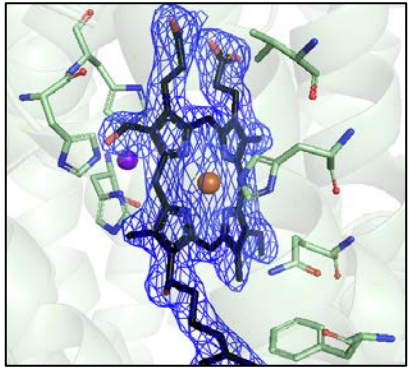
SSX structure of *ba3-type* CcO at 2.3Å
Data collected in 200um glass capillary
(BioMAX, MaxIV laboratory)



SSX structure of *ba3-type* CcO at 2.3Å
(Data collected in 200um glass capillary, BioMAX)

SSX structure of *ba3-type* CcO at 3.6Å
HVE injector (100um nozzle), BioMAX
Andersson R, Safari C, *et al.,* Acta Crystallogr D Strcut Biol., Oct 2019





SFX structure of *ba3-type* CcO at 2.3Å
LCP injector (75um nozzle), SACLA
Andersson *et al.* Scientific Reports, 7: 4518 (2017)

Courtesy to Swagatha Ghosh
Richard Neutze's Group

Serial X setup at ID23-2 beamline

Daniele D Sanctis, ESRF; Max Nanao, ESRF
Anton Popov, ESRF; Peter Van den Linden, CEA
Neutze Lab from University of Gothenburg

Summary

- SSX-based automated fragment screening using CrystalDirect
- Viscous injectors are available for interested users
- Development on capillary-based SSX sample-delivery with Neutze Lab is ongoing
  - Open to interested users for commissioning at the beamline

Thanks to ….

- ESRF
  - Gordon Leonard
  - Christoph Muller-Dieckmann
  - Daniele D Sanctis
  - Max Nanao
  - Gianluca Santoni
  - Sasha Popov
  - Marcus oscarson
  - Antonia Betleva
  - Anton Popov
  - Peter Van den Linden
  - David Flot
  - Igor Melnikov
  - Didier Nurritzo
- EMBL
  - Andrew McCarthy
  - Matthew Bowler
  - Jose A Marquez
  - Florine Dupeux
  - Anne-Sophie Humm
  - Instrumentation Team
- IGF-Montepellier
  - Sebastien Granier
  - Robert Healey
- University of Gothenburg, Sweden
  - Richard Neutze
  - Swagatha Ghosh

Our worldwide user community

EMBL

CrystalDirect™ – unique concept of automated harvesting

HTX Lab, J.A. Marquez

Open Access HTX Facility

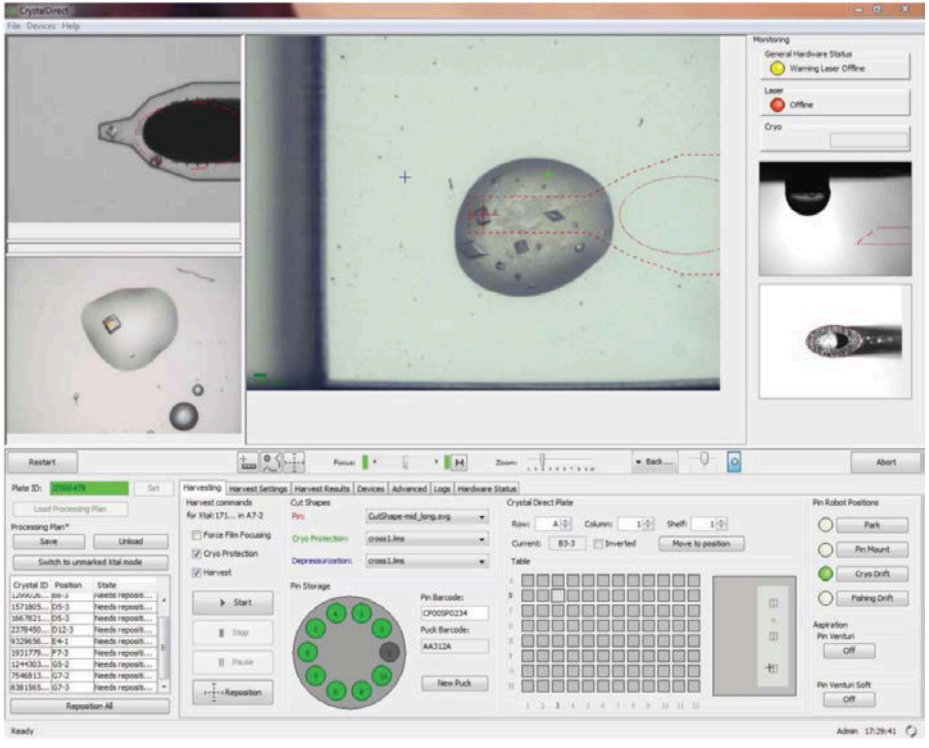
Access for European academic scientists funded through:

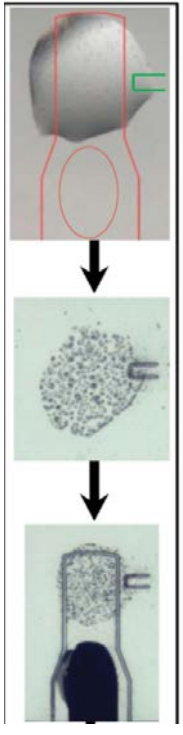
iNEXT Discovery

BioStruct-X

iNEXT

instruct Integrating Biology





Zander et al., *Acta Cryst D.* 2016

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