



FragMAX

Crystallographic fragment screening at MAX IV

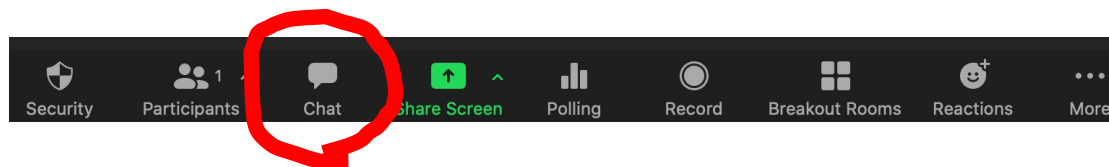
Webinar, 17/06/2021

Sandesh Kanchugal, Elmir Jagudin & Tobias Krojer

Outline

- MAX IV Laboratory
- Introduction to crystal-based fragment screening
- FragMAX components and design:
 - Crystal preparation workflow
 - Data collection at BioMAX
 - Large-scale data processing (including FragMAXapp demo)
- Prerequisites & crystal checklist
- Access modes for academia and industry
- Current & future developments
- Q&A

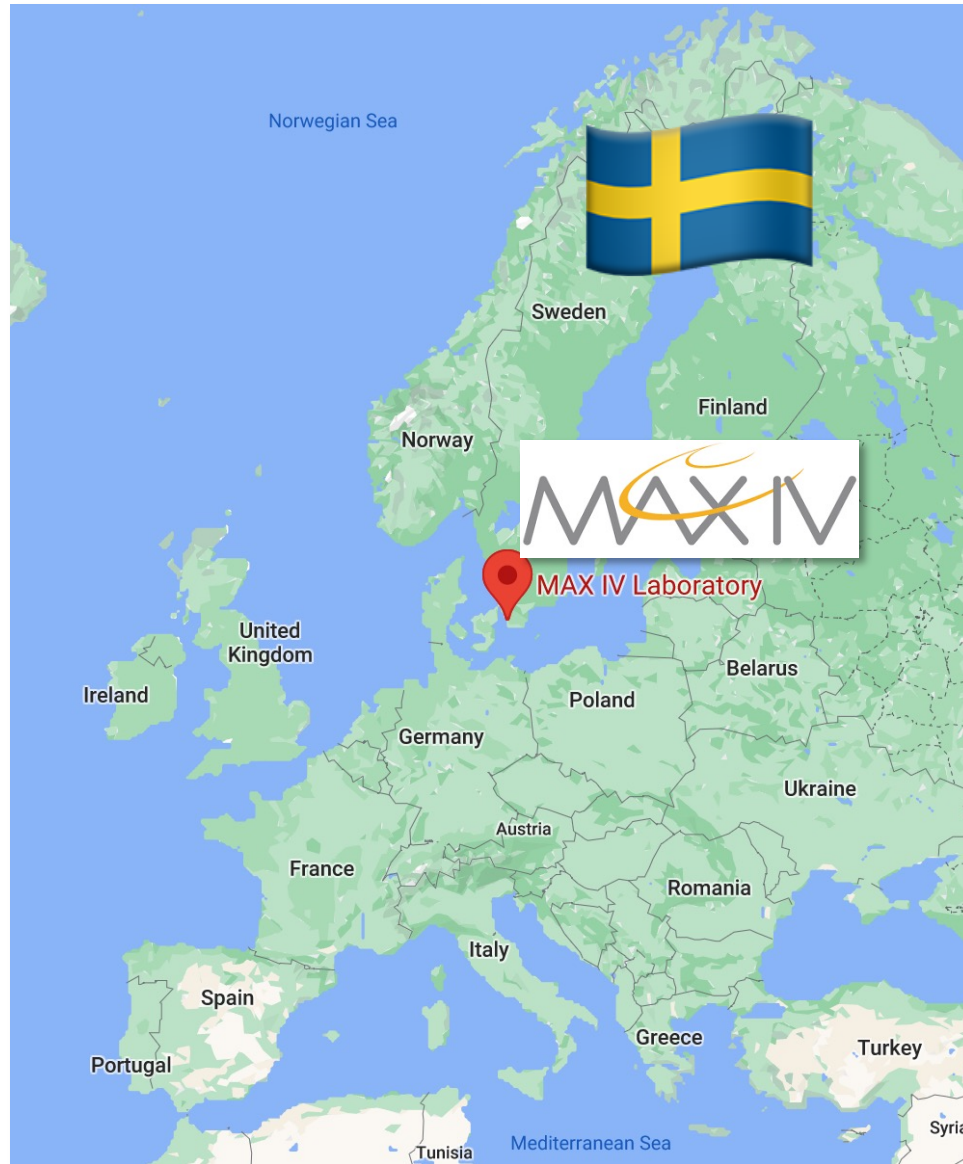
Use ZOOM Chat for questions or get in touch: tobias.krojer@maxiv.lu.se



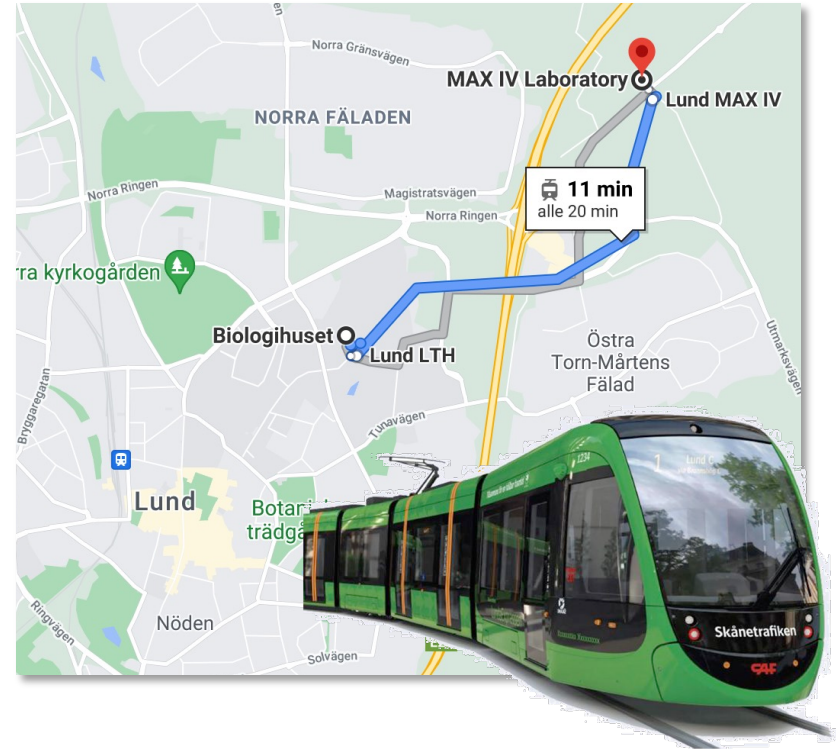
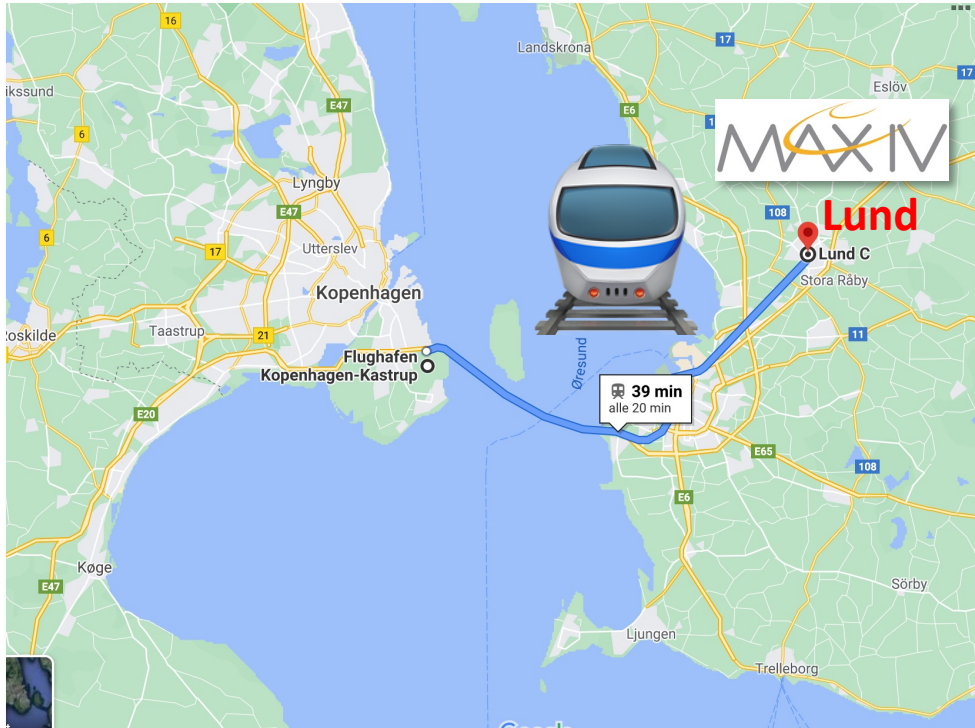
Download presentation from https://indico.maxiv.lu.se/e/FragMAX_webinar

MAX IV Laboratory

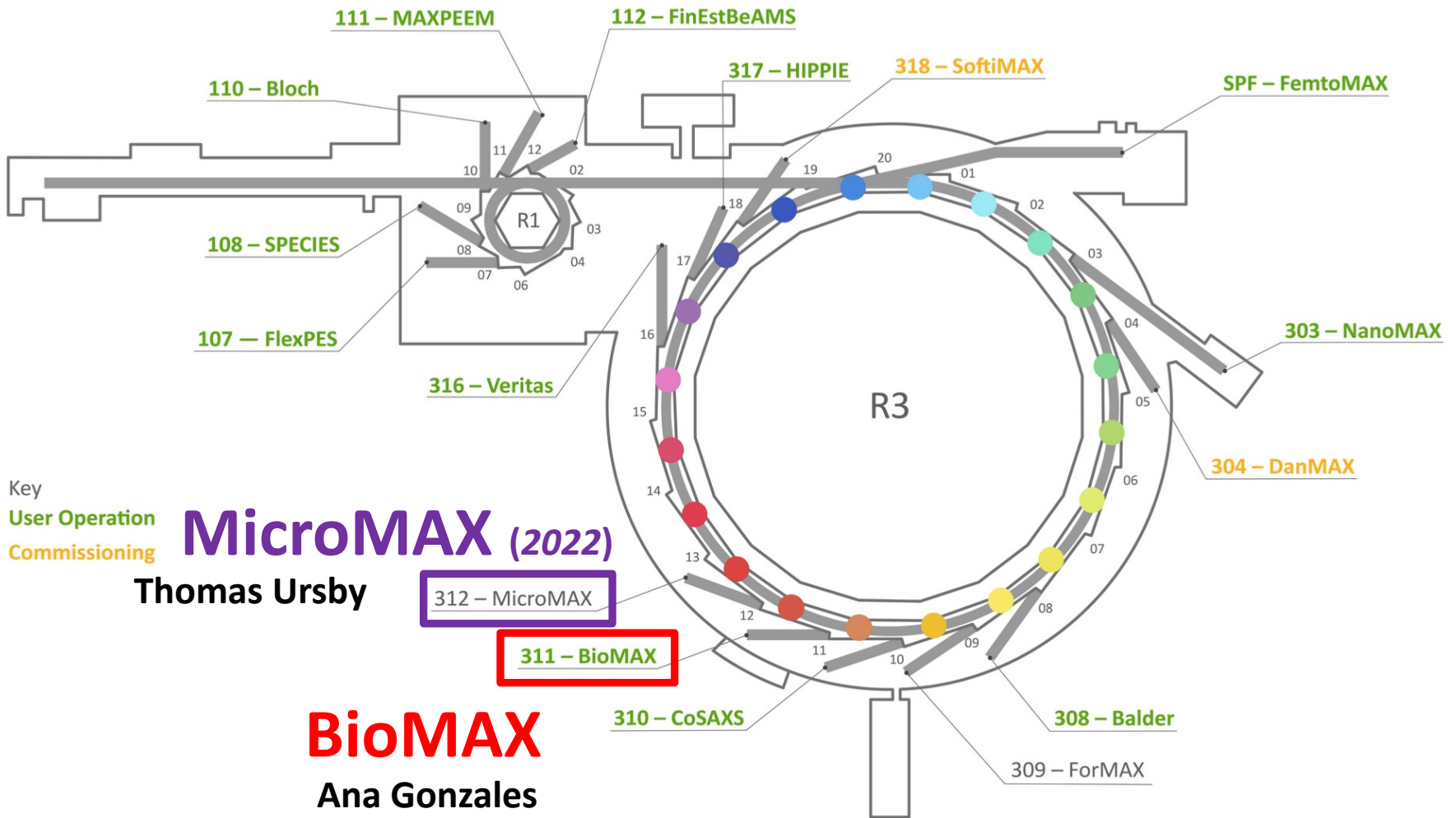
MAX IV Laboratory



MAX IV Laboratory



MAX IV Laboratory



The first 4th generation synchrotron in operation

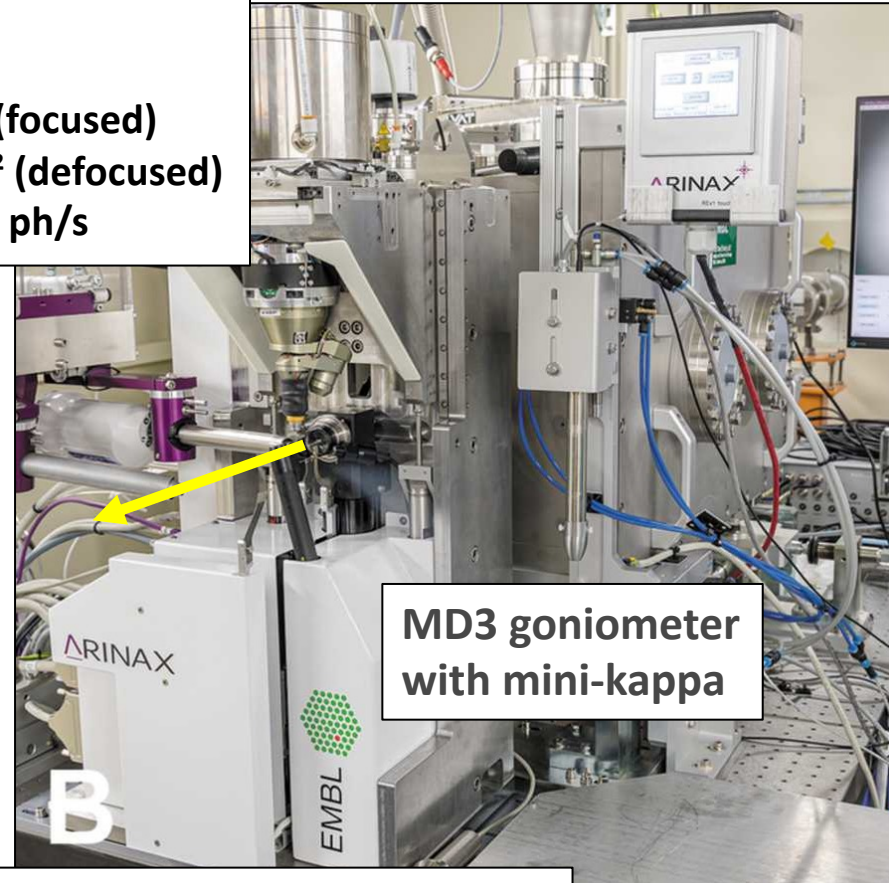
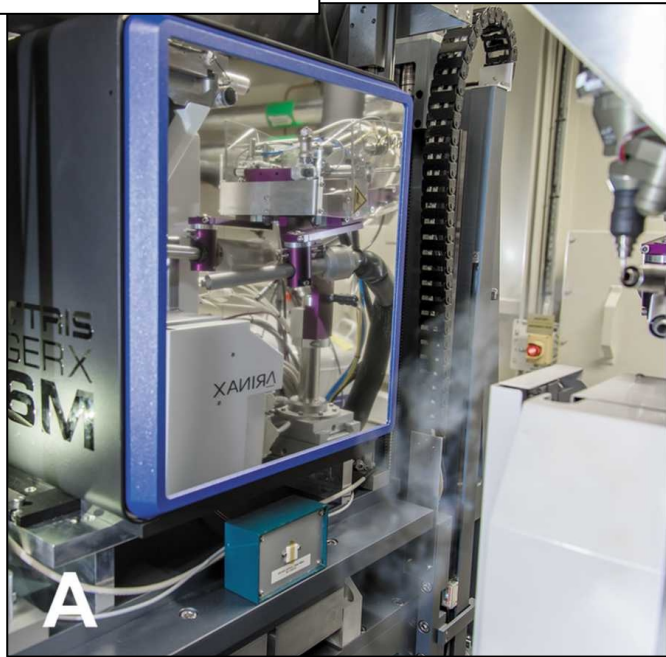
BioMAX beamline

<https://www.maxiv.lu.se/accelerators-beamlines/beamlines/biomax>

Beam:

- 5 - 25 keV
- $20 \times 5 \mu\text{m}^2$ (focused)
- $50 \times 50 \mu\text{m}^2$ (defocused)
- flux: $7 \cdot 10^{12}$ ph/s

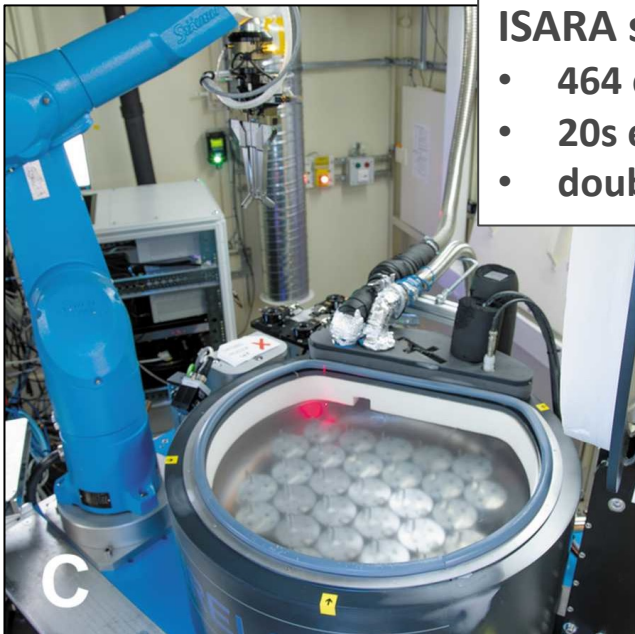
Eiger 16M detector



MD3 goniometer
with mini-kappa

Default exposure time:
11ms per 0.1 degree
→ 360 degree in < 40 seconds

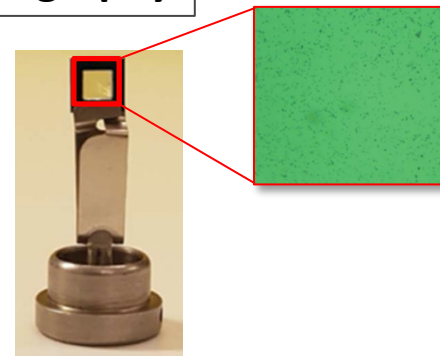
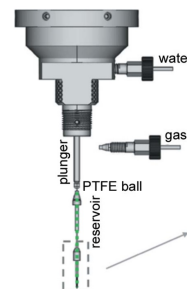
Data collection @ BioMAX



ISARA sample changer:

- 464 crystals
- 20s exchange time
- double-gripper

Serial crystallography



Shilova, A. et al. (2020). Current status and future opportunities for serial crystallography at MAX IV Laboratory. *J Synchrotron Rad* 27, 1095–1102.

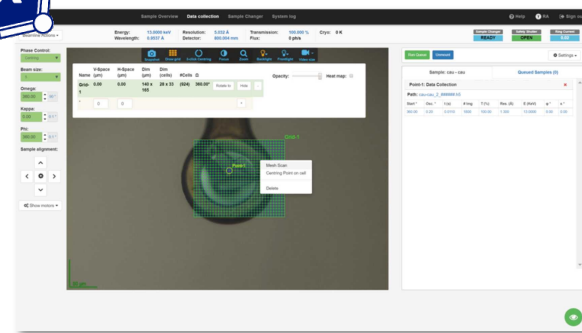
Remote operation



> 20 datasets per hour



MXCuBE3 user interface

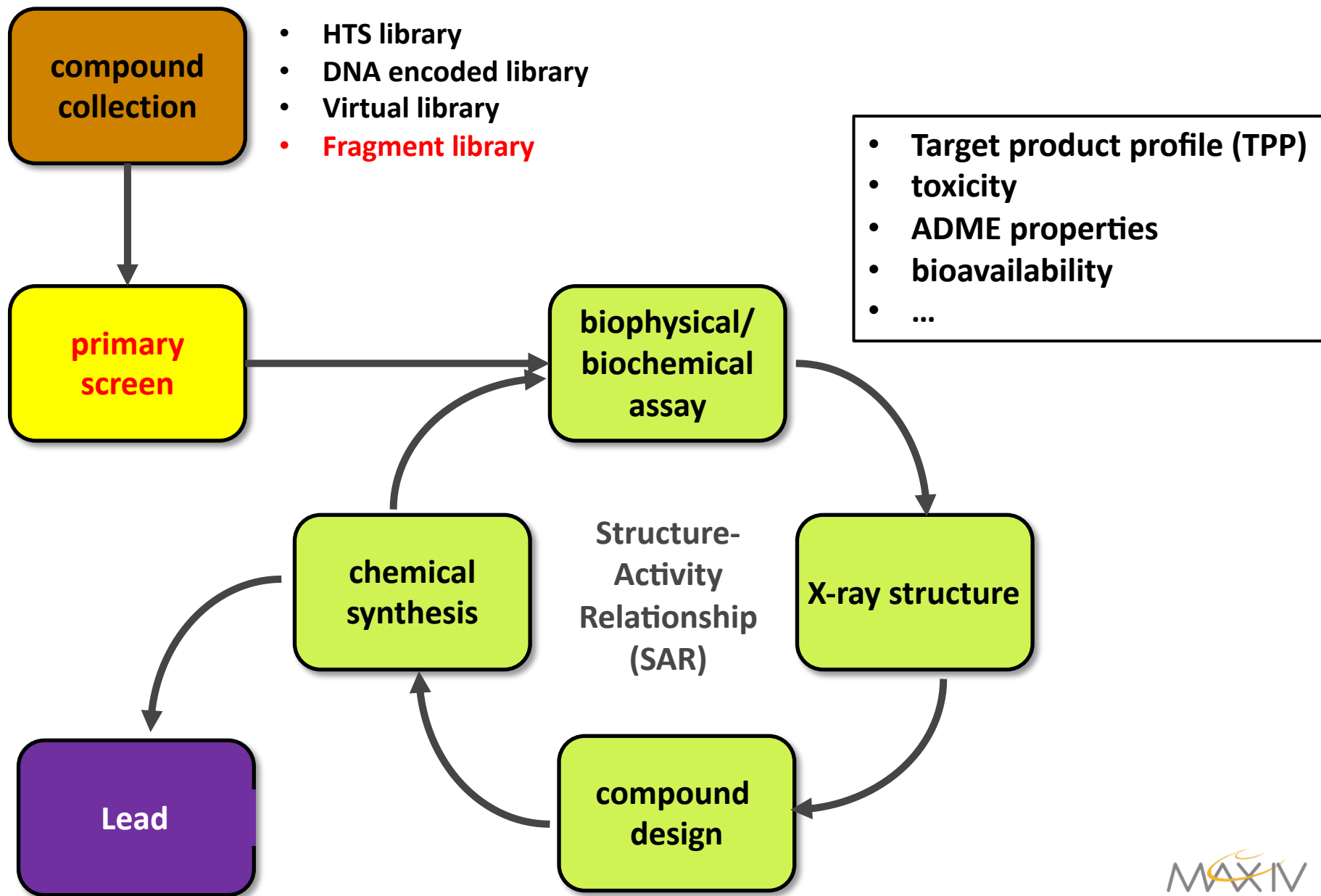


<https://www.maxiv.lu.se/accelerators-beamlines/beamlines/biomax/user-access/remote-experiments-at-biomax/>

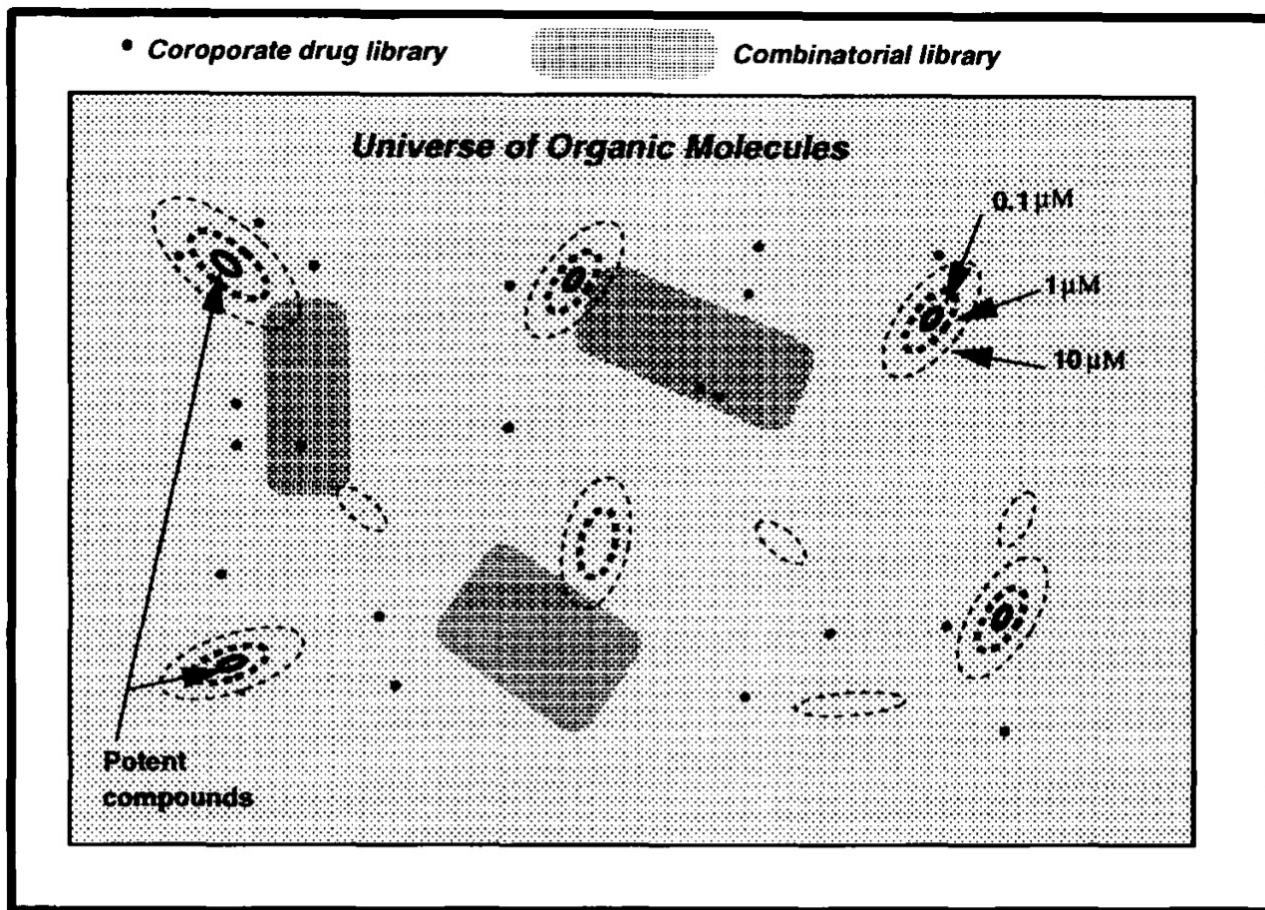
Ursby et al. (2020). BioMAX – the first macromolecular crystallography beamline at MAX IV Laboratory. *J Synchrotron Rad* 27.

Introduction to crystal-based fragment screening

Structure-based drug design



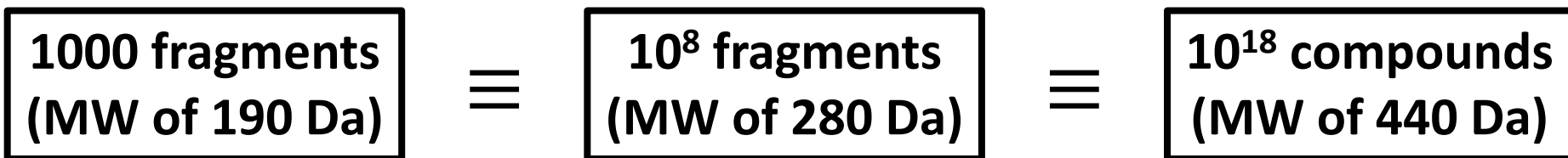
Number of drug-like molecules is enormous



10^{63} potential molecules... (???)

Fragment libraries cover vast regions of chemical space

“Equivalence” of chemical space:



HTS screening

- Searching for potent molecules
- Complex molecules \rightarrow Low probability



Millions
of complex molecules

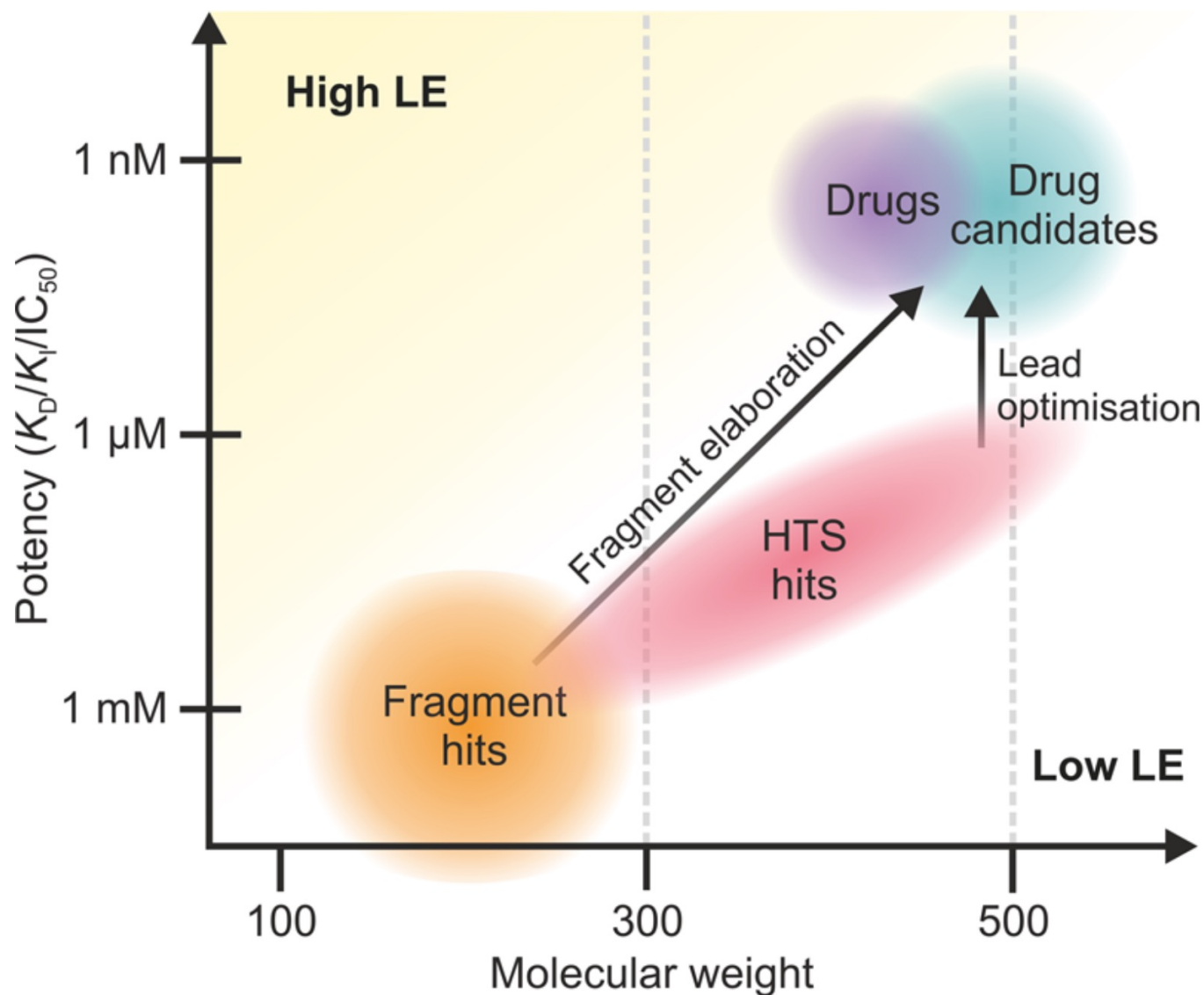
Fragment screening

- Guaranteed binding – but weak
- Potency through chemical elaboration

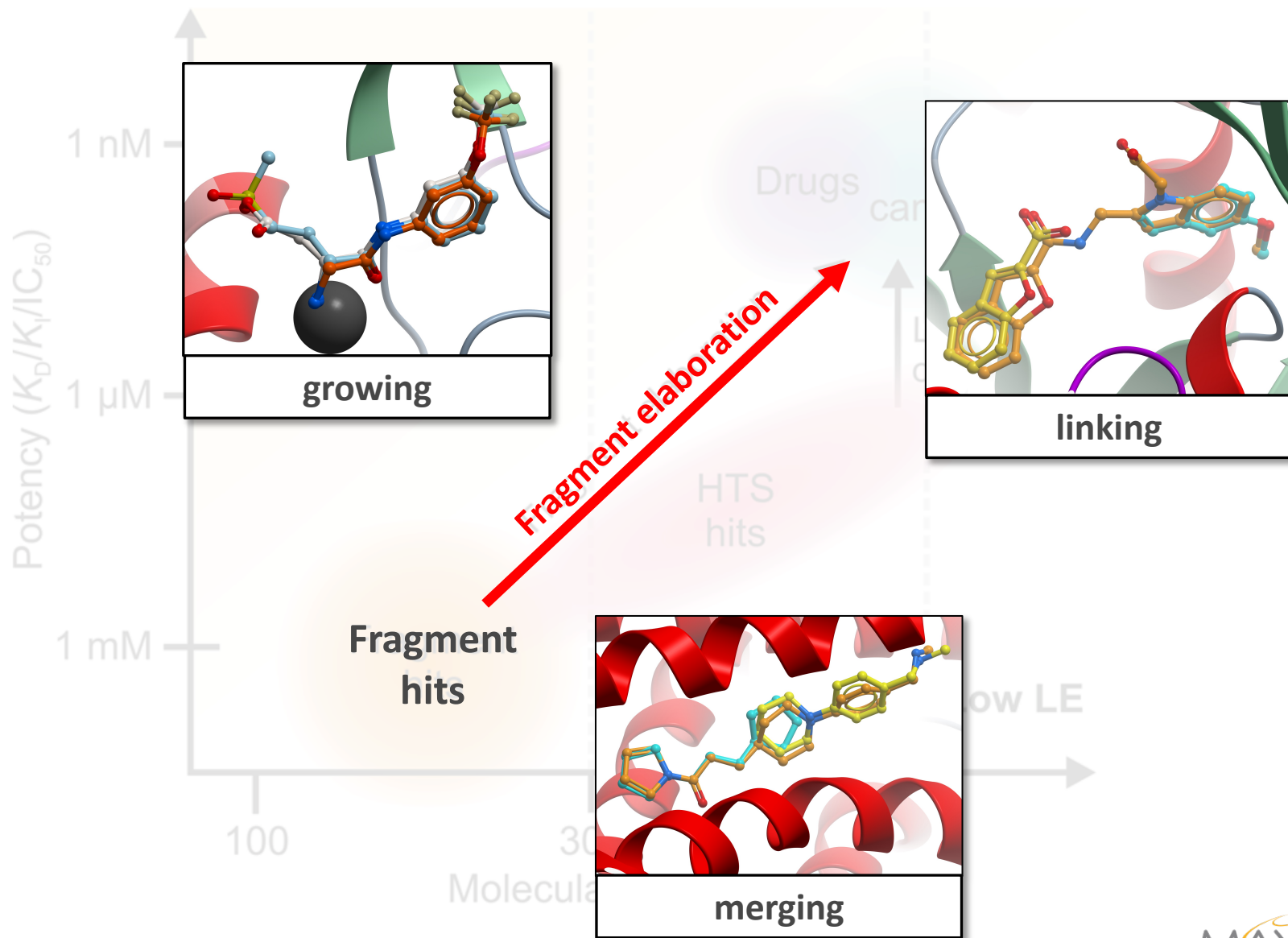


1000s
of small molecules

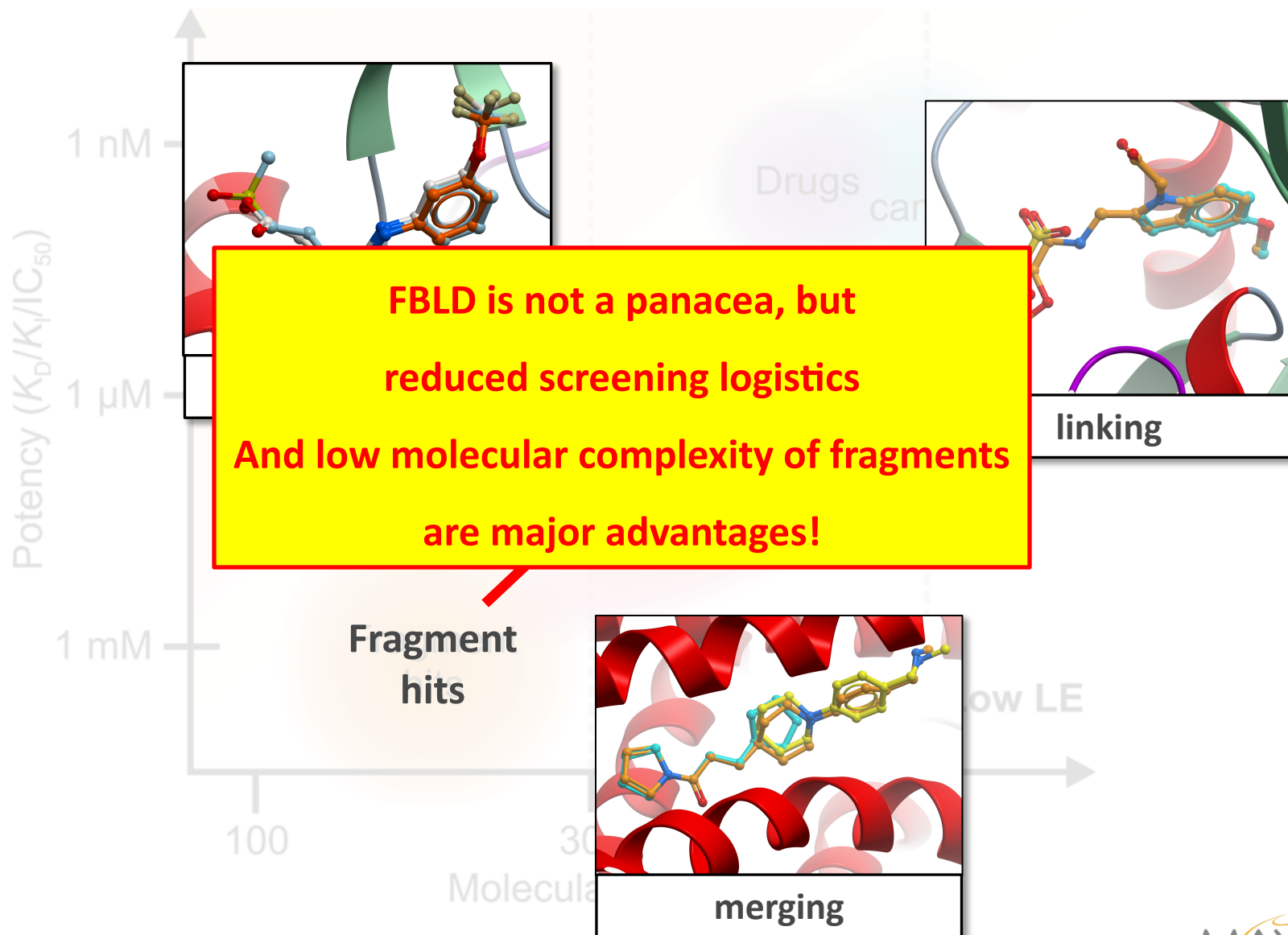
Principle of fragment-based lead development



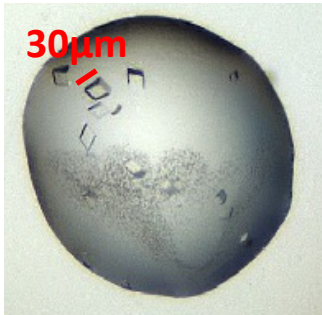
Principle of fragment-based lead development



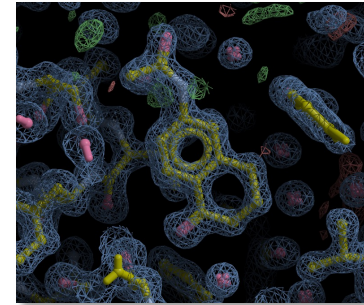
Principle of fragment-based lead development



Protein Crystallography as a primary screen!!!



100 nL protein : 100 nL reservoir
→ 1µg protein



- Limited sensitivity of most biochemical/ biophysical methods
- MX: high compound concentration (> 100mM); very sensitive!
- FBLD relies on structural information
- Protein Crystallography has seen huge improvements over the last decade
- Long history of how to share large-scale infrastructure
- Emergence of several crystallographic screening centers in Europe



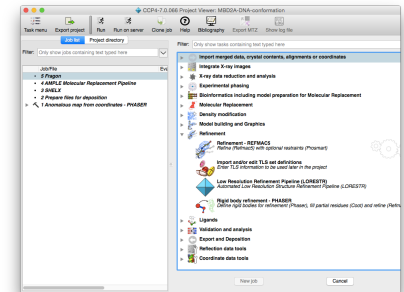
Detector



Robot



Beam



FragMAX - en plattform för high throughput screening av fragment i läkemedelsutveckling genom röntgenkristallografi



Vetenskapsrådet

SWEDISH RESEARCH COUNCIL



LP3 - Lund Protein
Production Platform



Uwe Müller
Gustavo Lima

Wolfgang Knecht

Derek Logan

Tove Sjögren



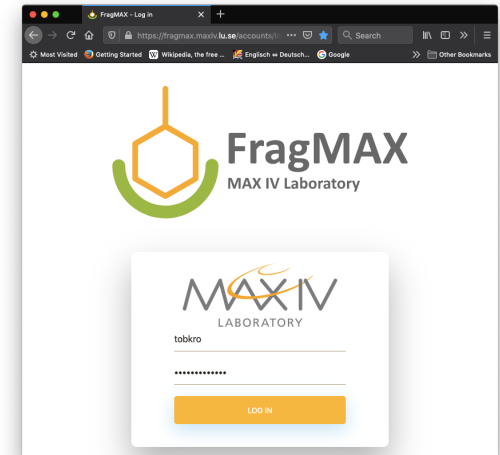
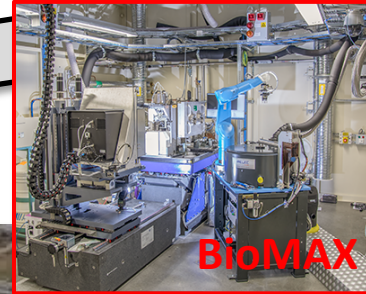
FragMAX components and design



LP3 - Lund Protein
Production Platform



Data Collection



Data Analysis

Crystal preparation

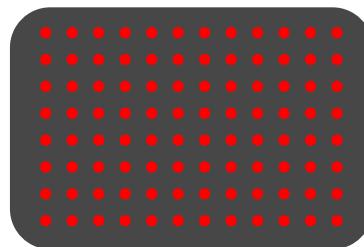
Lima et al. (2020). FragMAX: the fragment-screening platform at the MAX IV Laboratory. *Acta Cryst D* 76, 771–777.

Lima et al. (2021). FragMAXapp: crystallographic fragment-screening data-analysis and project-management system. *Acta Cryst D* 77, 799-808.

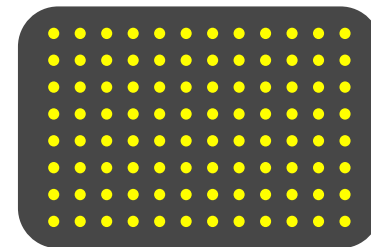
Crystal preparation @ LP3

> 1000 fragments available

- **FragMAXlib (172)**
- F2X entry (96)
- EU-OPENSOURCE (1000)
- MiniFragments (80)
- + bring your own*



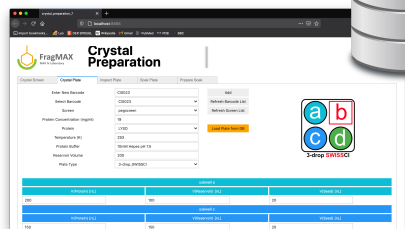
Compound plate



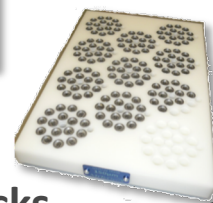
Crystal plate

robot-assisted crystal
soaking & mounting

project management
& database infrastructure



pins & pucks

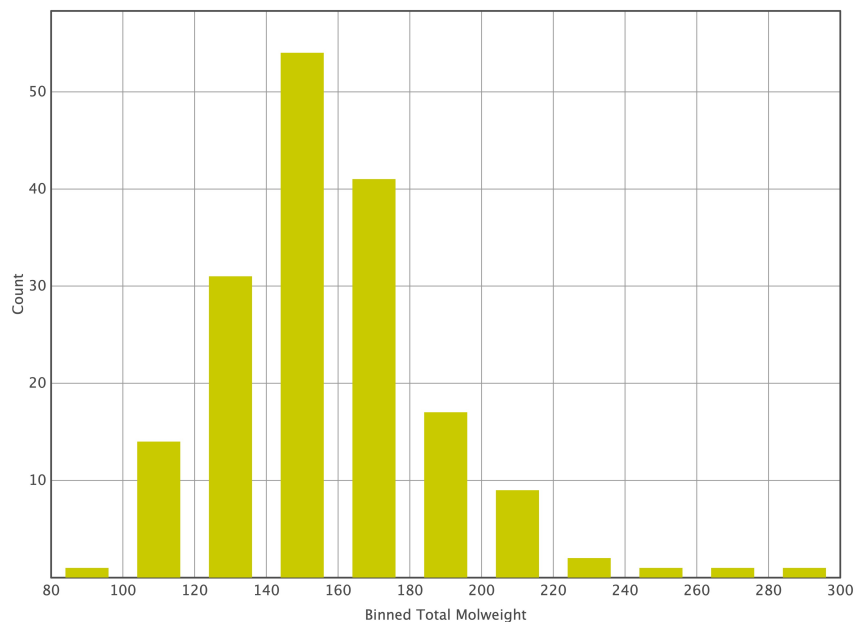


Crystal SHIFTER



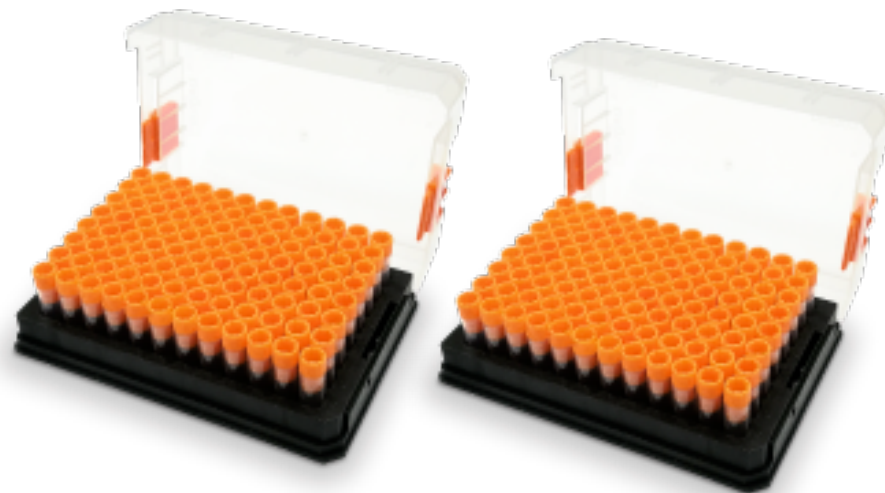
Compounds, equipment & consumables are free of charge!

New Fragment library @ FragMAXlib



Molecular weight (Da)

Descriptor	min	max	mean
Mw	108	269	158
Num. heavy atoms	8	18	11.2
SlogP	-0.7	2.6	1
Num. H-bonds acc.	1	5	2.5
Num. H-bonds don.	0	2	0.9
Num. rot. bonds	0	3	1



172 compounds



Shipment possible!

Fragment Libraries

<https://www.maxiv.lu.se/fragmax/fragmaxlib>

FragMAXlib

- 170 compounds
- DMSO, EG, powder

F2X entry

- 96 compounds
Wollenhaupt, J. *et al.* F2X-Universal and F2X-Entry: Structurally Diverse Compound Libraries for Crystallographic Fragment Screening. *Structure* 28, 694-706.e5 (2020).

Frag Xtal Screen

- 96 compounds
Huschmann, F. U. *et al.* Structures of endothiapepsin–fragment complexes from crystallographic fragment screening using a novel, diverse and affordable 96-compound fragment library. *Acta Cryst F* 72, 346–355 (2016).

EU-OPENSREEN fragment library

- ca. 1000 compounds + 80 MiniFragments
- In DMSO

Data processing & analysis @ FragMAXapp



Prerequisites & crystal checklist

Prerequisites

- **Most projects fail or get delayed at the crystallization stage!**
- reliable and reproducible crystallization is a must
- Crystal preparation at LP3 is optimized for 96-well SWISSCI sitting drop plates
- default FragMAX protocols are based on crystal soaking, i.e. “site of interest” must to be accessible (‘soakable’)
- robust crystals
- ‘big’(ish), 3-dimensional crystals
- Crystals should reliably diffract $< 2\text{\AA}$
- Crystal soaking & mounting at FragMAX is done at room temperature
- Crystals need to be tolerant to DMSO (or Ethylene glycol)

Considerations

- Understand your crystal system before you start your experiment
- Understand your structure
- Assumption: all crystals are isomorphous
- Multiple crystal forms
- Build the best possible reference model
- Screening campaign involves screening of approx. 250 compounds

Facility usage models

#3 Crystal prep (User – LP3), Data collection (User)

Users come on site to prepare crystals at LP3 for subsequent (remote) data collection

Post-Covid and not before 2022!

#1 Crystal prep (FragMAX team), Data collection (User - remote)

Users send ready-to-crystallize protein (40ul aliquots)

+ crystallization reagents (solutions, seeds etc.)

+ crystallization protocol

#2 Crystal prep (User – home lab), Data collection (User - remote)

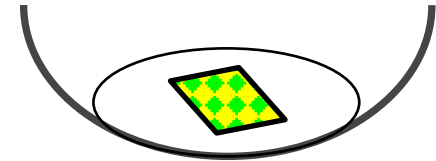
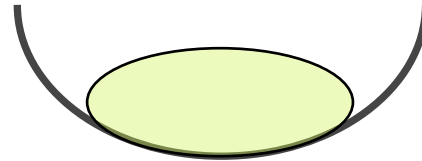
FragMAX team sends FragMAXlib, User prepares crystals and sends back to MAX IV

Principle of crystal-based fragment screening

Co-crystallization

Protein

Fragment



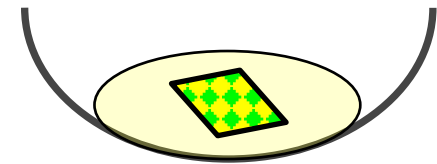
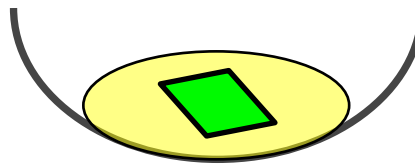
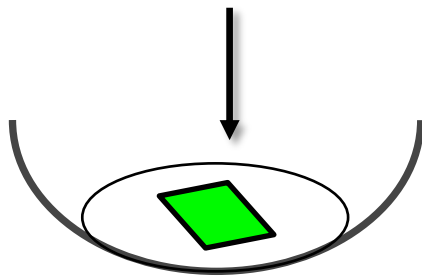
+ easy to automate

- Low fragment concentration

- many false negatives

Soaking

Fragment



- Requires complex liquid handling

+ very high fragment concentration

Standard soaking procedure at FragMAX

e.g.

100 nL fragment solution (in DMSO)
+ 900 nL crystallization solution



→ Incubate for several hours or overnight before mounting

→ 'dip soaks' are possible if necessary

Solvent characterization at home lab

Perform solvent characterization before screening campaign!

1. Establish baseline diffraction limit under optimal circumstances
2. Test diffraction of at least 3 crystals at each concentration:
 - 5% EG/ DMSO
 - 10% EG/ DMSO
 - 15% EG/ DMSO
 - 20% EG/ DMSO
 - 25% EG/ DMSO
 - 30% EG/ DMSO

Crystal preparation @ LP3

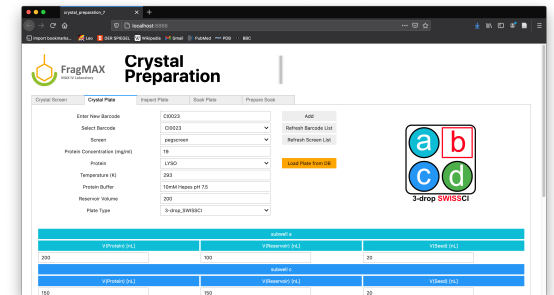
1. Protein crystallization



SWISSCI
Drop volume:
100 – 500 nL

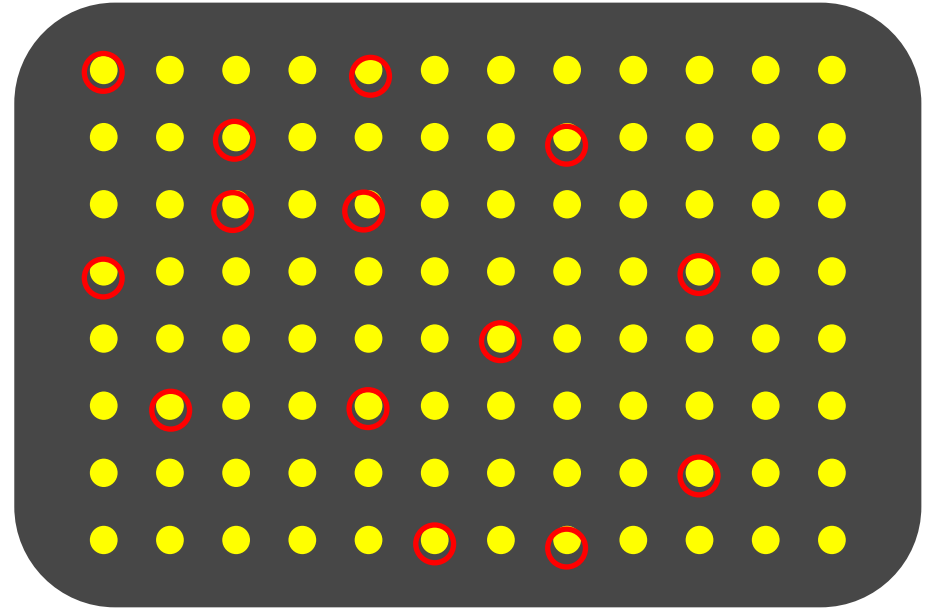
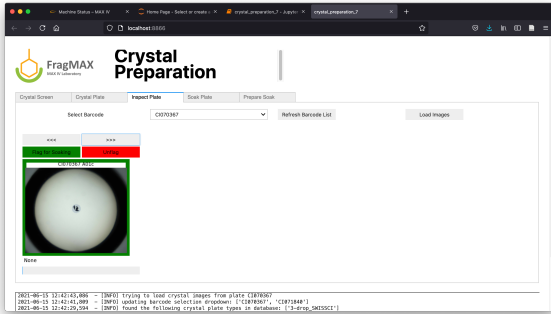


4°C & 20°C

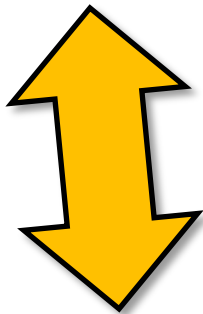


Crystal preparation @ LP3

2. Crystal selection

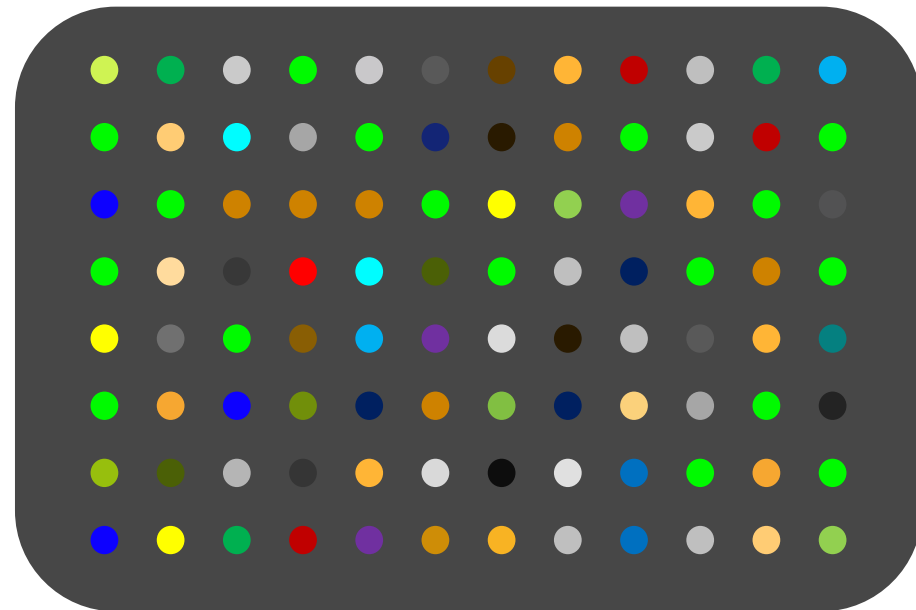
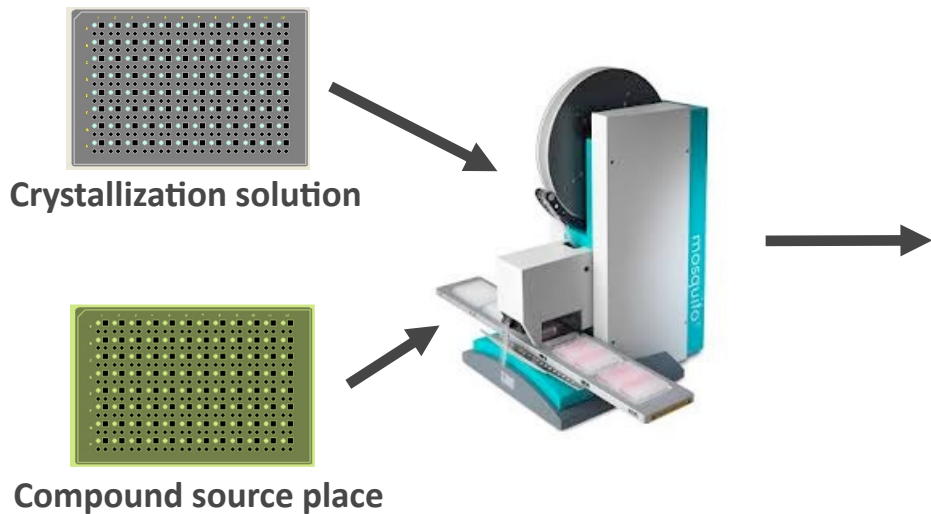


Crystal plate

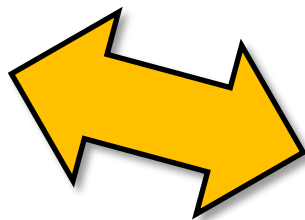
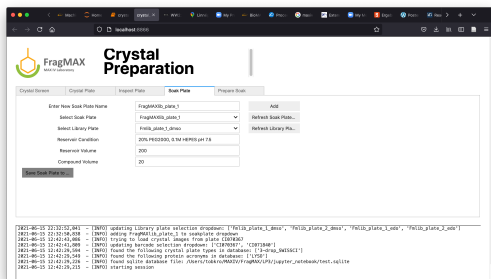


Crystal preparation @ LP3

3. Compound plate preparation

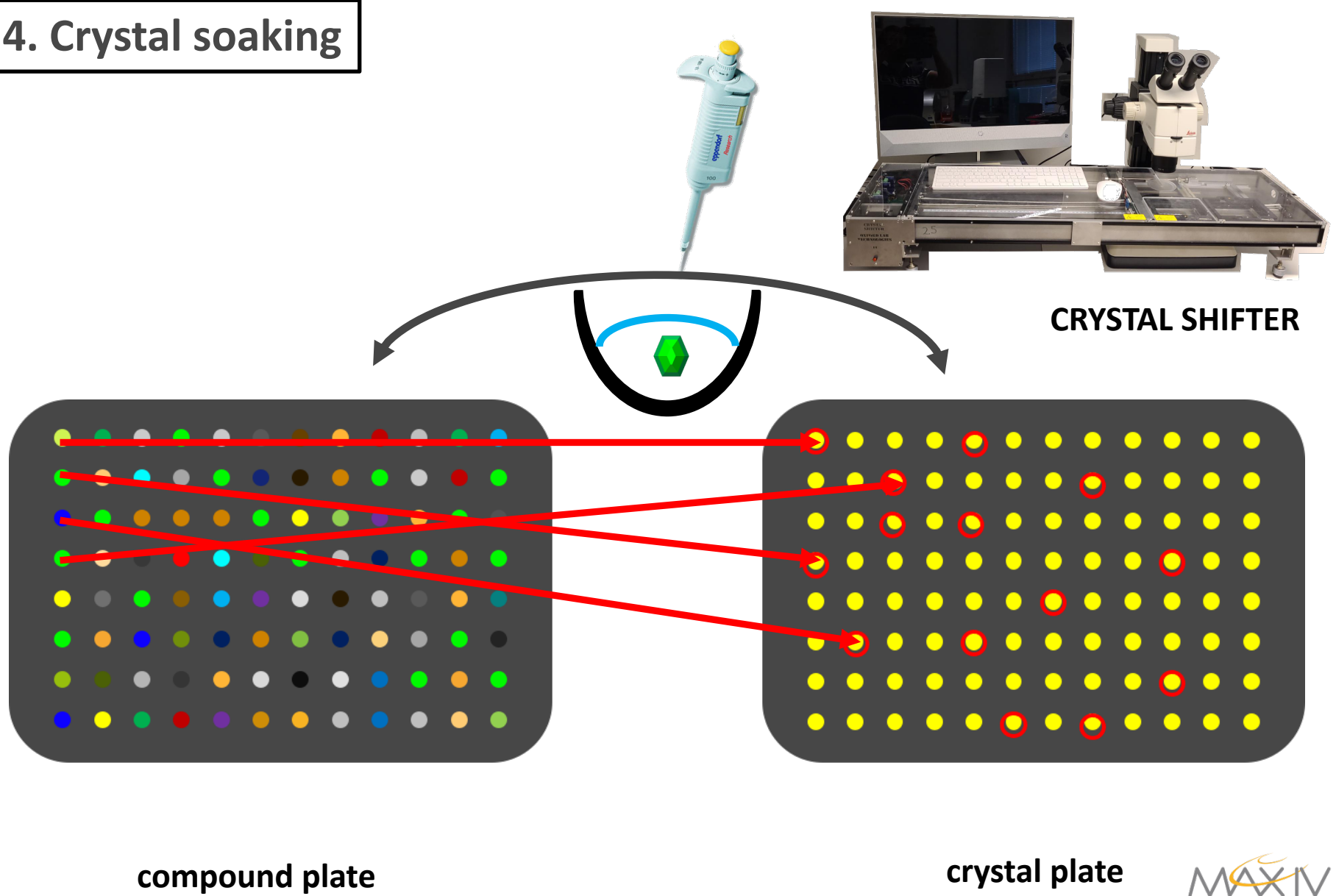


Compound plate



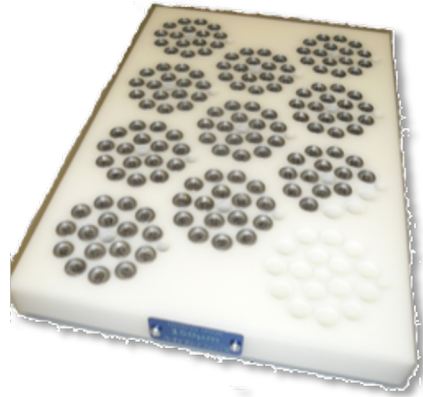
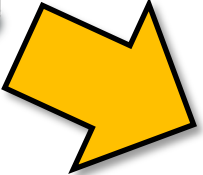
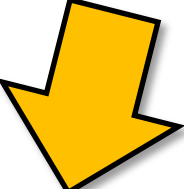
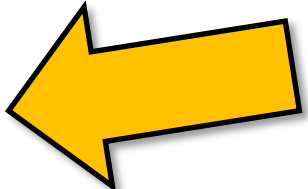
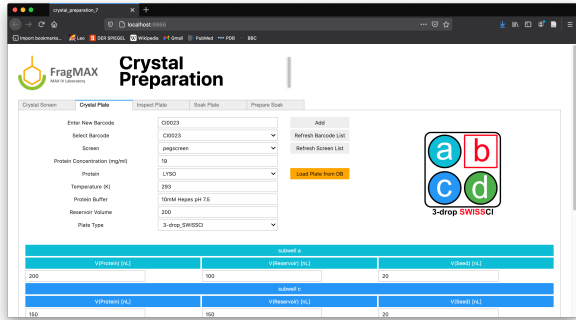
Crystal preparation @ LP3

4. Crystal soaking

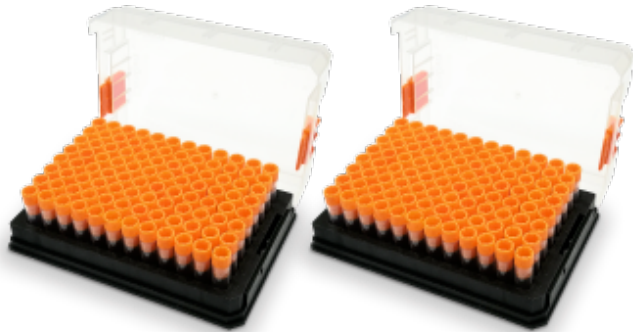


Crystal preparation @ LP3

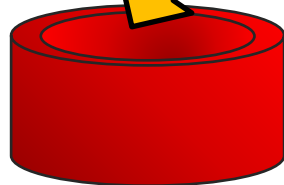
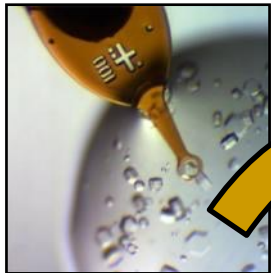
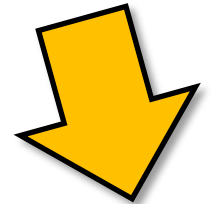
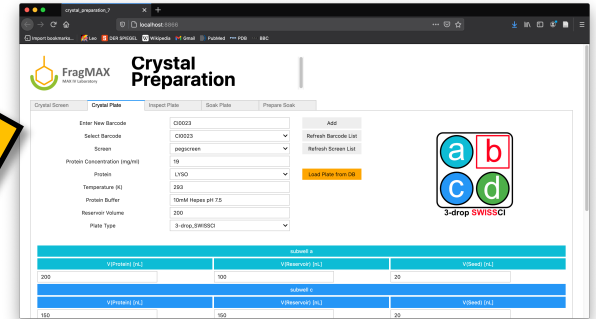
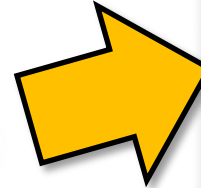
5. Crystal mounting



Crystal preparation @ home



FragMAXlib



Home lab



MAX IV



Current workflow

	Location	Status
Crystal Preparation		
Crystallization	LP3/ home lab	available
Soaking	LP3/ home lab	available
Mounting	LP3/ home lab	available
Data Collection		
manual	BioMAX	available
automatic	BioMAX	not available
Data Analysis		
Data Reduction	FragMAXapp	available
Initial Refinement	FragMAXapp	available
Compound restraints	FragMAXapp	available
PanDDA		available
analyse	FragMAXapp	available
inspect	MAX IV thinlink client/ home lab	available
Refinement	Home lab	<i>in progress</i>
PDB deposition	FragMAXapp	<i>planned</i>

Current status

- Several internal screening campaigns
- 1st Proprietary screening campaign May 2020
- 1st Long-term proposal screening campaign November 2020
- MAX IV summer shutdown: July 10th – September 12th

Access modes for academia and industry

Access modes



GET IN TOUCH



Academic users:

FragMAX Team

+46 46 2226779

tobias.krojer@maxiv.lu.se

Industrial users:

Industrial Relations Team

+46 725 546 309

magnus.larsson@maxiv.se

Access modes

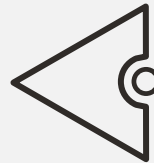
1. PEER REVIEWED ACCESS

- Open calls twice per year
- Apply for beamtime
- Proposals ranked on scientific merit
- Beamtime and lab usage for free

NEED TO PUBLISH

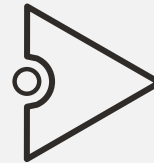
Access modes

TWO MAIN ROUTES



PEER REVIEWED
FREE ACCESS

PROPRIETARY
PAID FOR
ACCESS



MAXIV

2. PROPRIETARY ACCESS

- Short term – fast access
- Long term – framework agreement
- Confidentiality
- Fees for beamtime and services

RESULTS BELONG TO BUYER

Access modes

<https://www.maxiv.lu.se/fragmax/fragmax-user-information>

FragMAX user information

User access mode

Commercial/ Industrial access

Commercial or industrial user should contact MAX IV industrial relations team. More information is available on [this page](#).

Academic/ Open User Access

Currently, user access requires a regular project application during MAX IV [open calls](#).

Information about FragMAX applications:

- FragMAX applications will run as Longterm projects, guaranteeing access for followup projects that may come from the outcomes of a first screen.
- Users must fill a special application form and provide additional information (e.g. PDB structures, MSDS, etc).
- FragMAX runs as a scientific collaboration between the user group and FragMAX staff involved in the project execution, leading to co-authorship in scientific publications as part of MAX IV General Terms and conditions for Open User Access

Information about FragMAX template, user policies and MAX IV General terms and conditions are available in [this page](#).

<https://www.maxiv.lu.se/users/proposal-calls/longterm-calls-and-programs>

FragMAX – BioMAX Fragment Screening platform

You will find technical details on the [FragMAX website](#). FragMAX projects are conducted in a collaboration between the users / proposers and the FragMAX team at MAX IV, for details see proposal template. By submitting a proposal, proposers agree to the conditions for publications of results and ownership of IP (See special FragMAX template and [MAX IV user policies](#)).

How to submit a Longterm proposal, for FragMAX, in DUO

1. Make sure there is an open [proposal call](#) accepting Longterm proposals.
2. FragMAX proposals shall be discussed with the beamline team before submission and can be rejected if this is not done
3. Choose Proposal Type **Longterm**
4. Choose Research Area Structural Biology, Beamline **BioMAX**.
5. Use the special [special FragMAX template](#).

Please make sure to always upload all publications resulting from experiments at MAX IV to the [DUO Publication Database](#) and to link them to the relevant proposal ID and Beamline.

Rapid access

<https://www.maxiv.lu.se/users/proposal-calls/fast-access>

How to submit a Fast Access proposal in DUO

1. Make sure there is an open **proposal call** accepting Fast Access proposals.
2. Choose Proposal Type **Fast Access**
3. Choose Research Area according to your beamline of interest.
4. Use the **Fast Access Template**.

tbc...



Access modes

<https://inext-discovery.eu/network/inext-d/home>



About

Services

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Login

Apply for Access

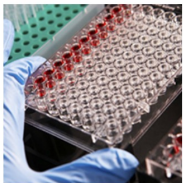
<https://inext-discovery.eu/submit-proposal/step1>

Submit a proposal for access

Please fill in the following fields describing your project and your needs. Fields marked with * are mandatory



Select what you want to apply for access to:



Signature Access

Select FBLD

Fragment/Ligand Screening

Ligand screening and fragment-based lead discovery by X-ray crystallography or NMR, in integrated work flows at different partner sites.

Select Centre



Fragment Screening, MAX IV, Lund, Sweden

Industrial Relations Office

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MAXESS Industry Arena is an evolving national initiative supporting and facilitating industrial use of the large-scale research infrastructures MAX IV and ESS, and the associated eco-system. MAXESS Industry Arena facilitates partnerships between experts and industrial users through maxess.se, case studies, networking events as well as guided introductions to the industrial advantages of neutron and synchrotron tools.

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How biological drug development benefits from photons

Alligator Bioscience in collaboration with SARomics Biostructures used X-ray crystallography to unveil the unique characteristics of one of their antibody-drug candidates, proving how valuable synchrotron X-rays can be for drug development.

A promising antibody

Alligator Bioscience, located just a few kilometres away from MAX IV, is a biotechnology company specialised in the development of antibody-based immunotherapies for cancer treatment. The company has a portfolio of different antibodies in development, one of these being ATOR-1017. The development of such macromolecular drug candidates for immunotherapy treatments is a complex and challenging business. Thorough and solid knowledge of the molecular structure and mode of action is crucial to identify the most promising candidates.



Through a collaboration with the CRO SARomics Biostructures and the use of synchrotron X-rays, Alligator successfully strengthened its knowledge on two of its antibody-drug candidates, including ATOR-1017. Using high-resolution X-ray crystallography, Alligator's researchers observed that ATOR-1017 binds its target at a unique site, a feature that sets it apart from competitor drug candidates. These findings make ATOR-1017 stand out as a promising antibody for the development of immunotherapies against cancer and serve as proof of the value that synchrotron light can bring to drug development.

Understanding the mechanism of action

PHARMA AND BIOTECHNOLOGY

Case Details

Oct 2018 - Mar 2020

Industrial user: Alligator Bioscience AB

Research partner

BioMAX

SARomics Biostructures

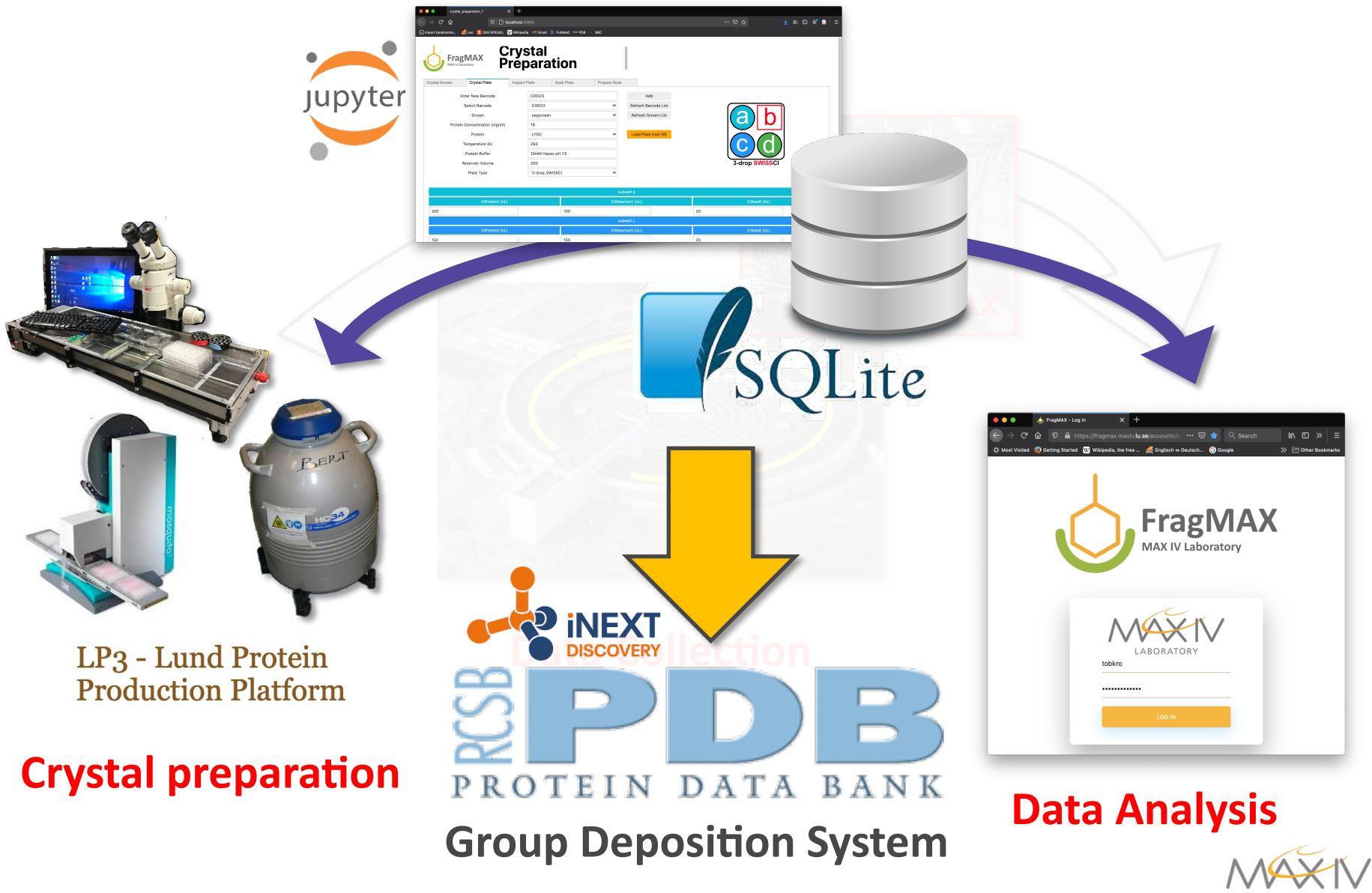
Source Files

Verification of the unique functionality of ATOR-1017 by 3D structure determination.pdf

MAX IV

Current Developments

NEW: DB implementation & meta-data capture



LP3 - Lund Protein Production Platform

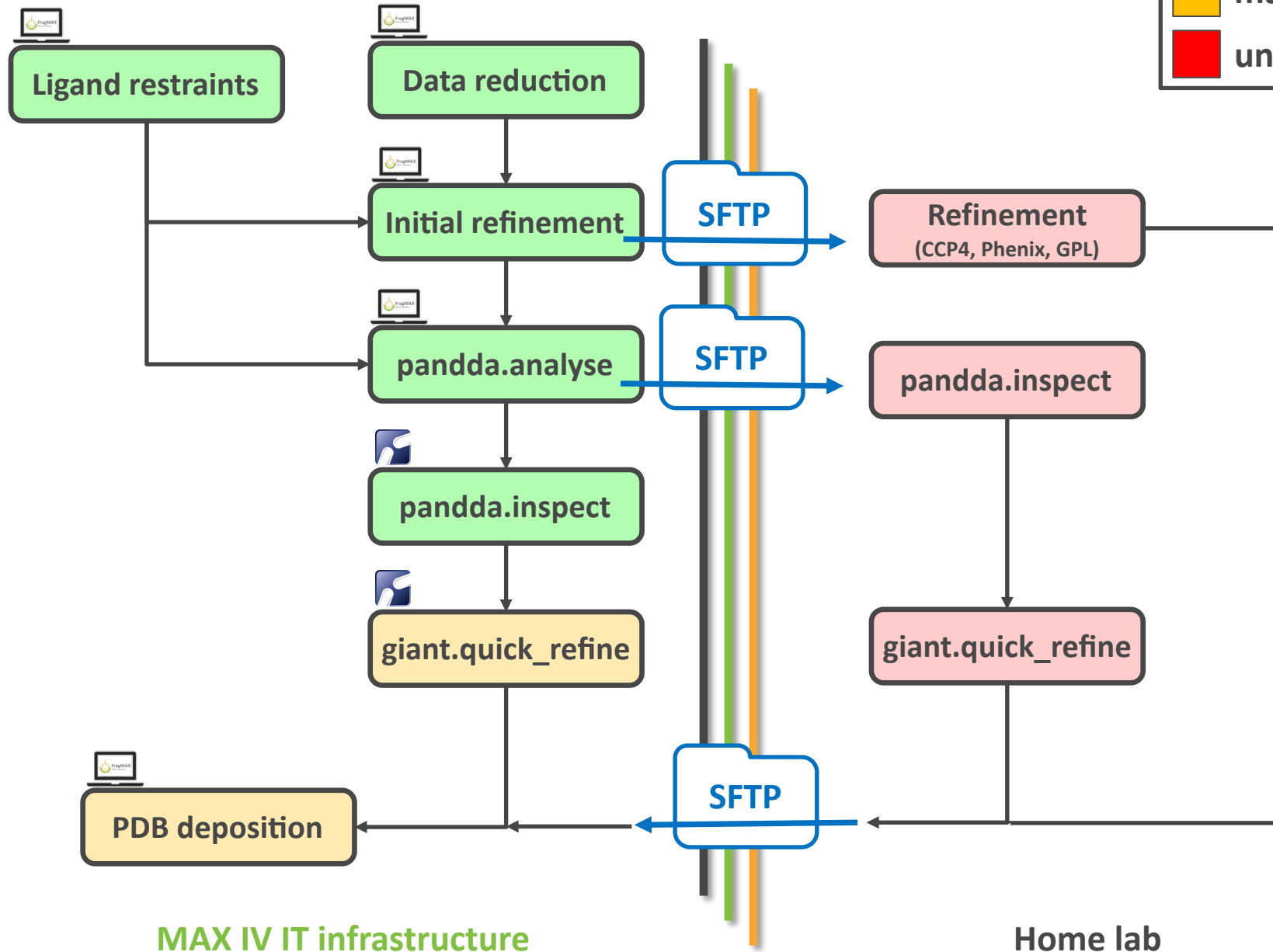
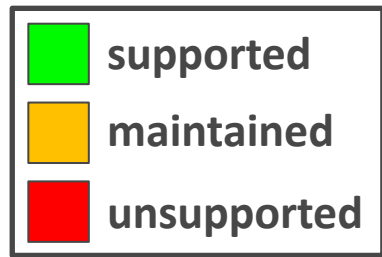
Crystal preparation

RCSB PDB PROTEIN DATA BANK Group Deposition System

Data Analysis



Refinement & Deposition support

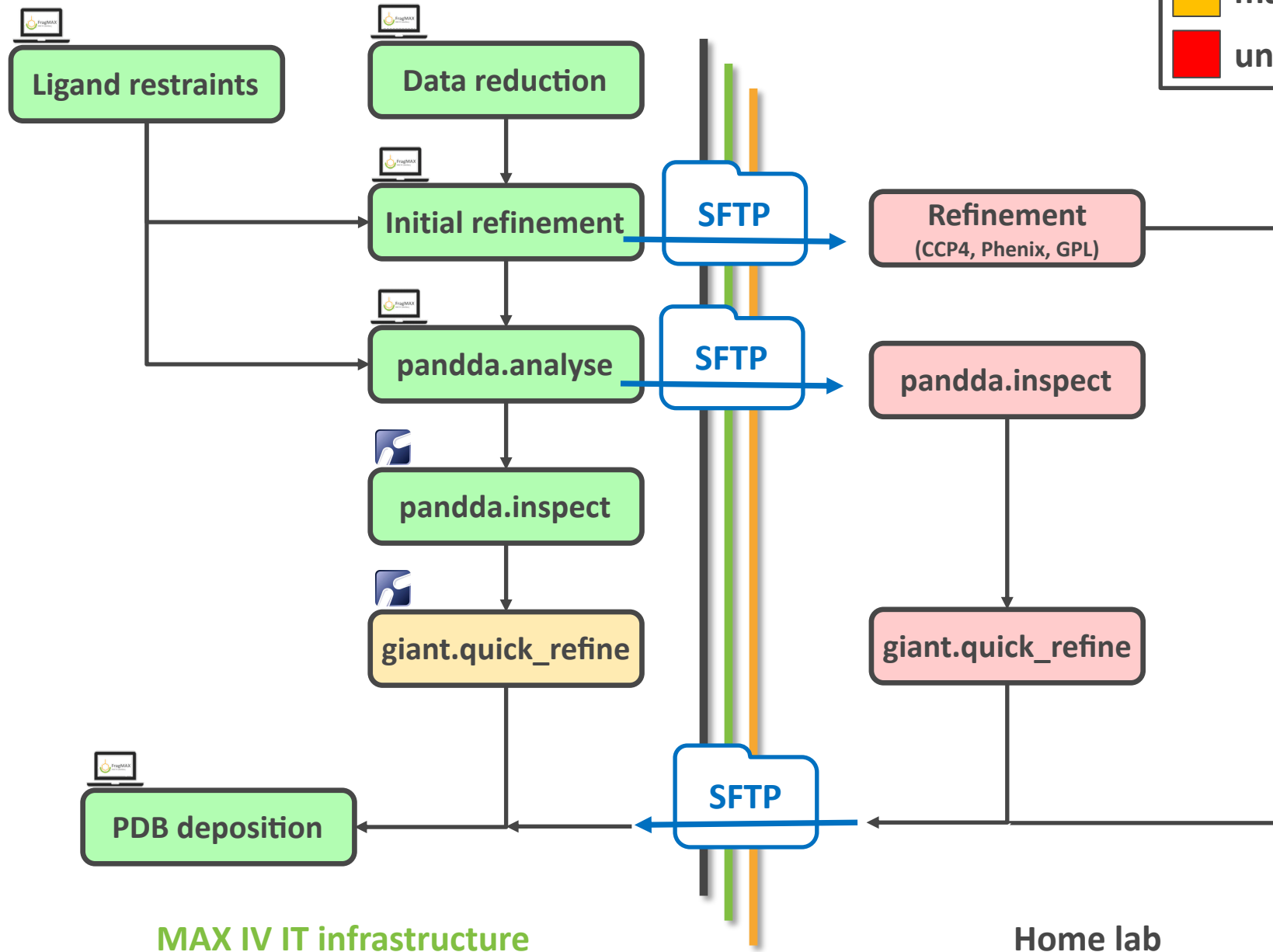
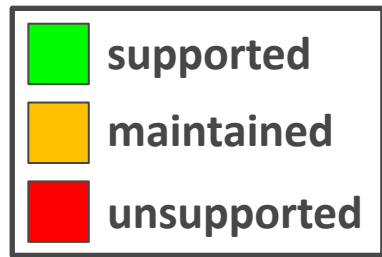


MAX IV IT infrastructure

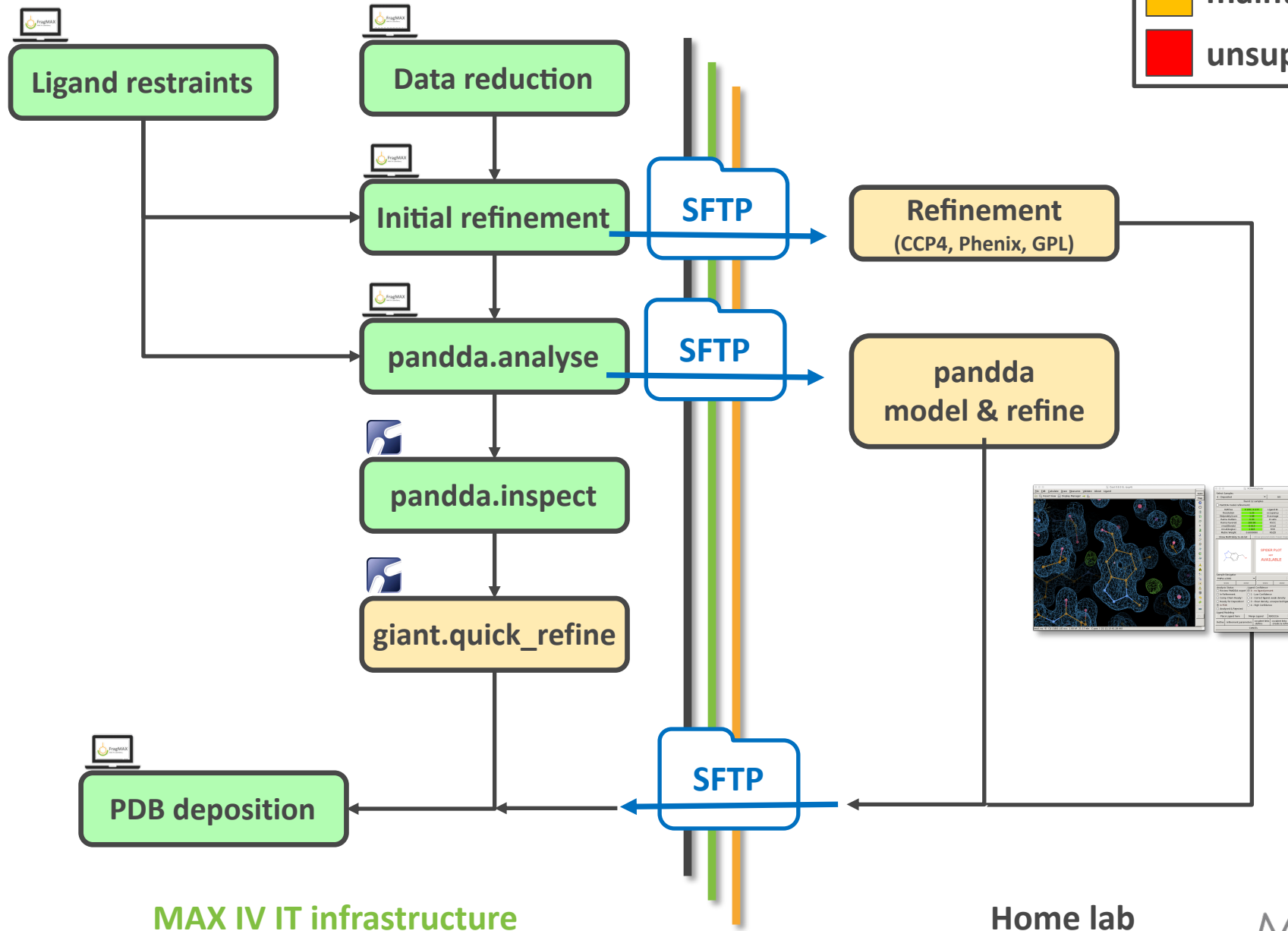
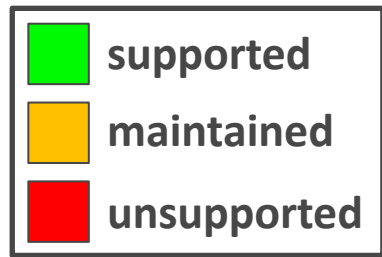
Home lab



Refinement & Deposition support



Refinement & Deposition support



MAX IV IT infrastructure

Home lab

Furthermore...

- Enhanced automation during crystal preparation
- Automated data collection...
- (Simplified fragment elaboration)

Summary

- **FragMAX is open for business!**
- **Alternative workflows?**
- **FragMAX beyond FBLD?**
- **New ideas?**
- **Get in touch if you are interested!**



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