FragMAX – BioMAX fragment screening platform at MAX IV

04/02/2022

Tobias Krojer

Fragments as starting points for drug development

- Development of small molecule drugs requires suitable starting points
- High-throughput screening (HTS) is only available to big pharma companies
- HTS libraries with several million compounds cover only a tiny part of chemical space
- Fragment screening provides a resource friendly & powerful alternative

HTS screening

- *Searching for potent molecules*
- *Complex molecules → Low probability*

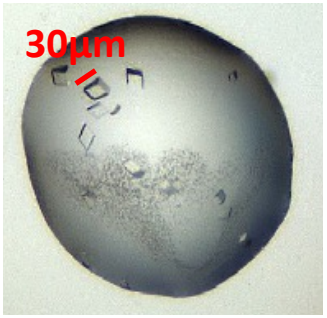
Millions
of complex molecules

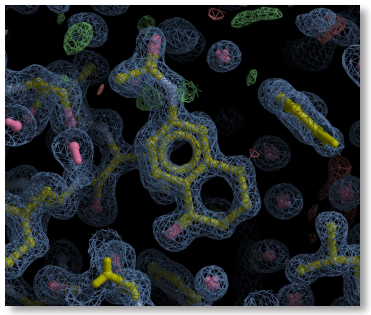
Fragment screening

- *Guaranteed binding – but weak*
- *Potency through chemical elaboration*

1000s
of small molecules

Protein crystallography for primary fragment screening



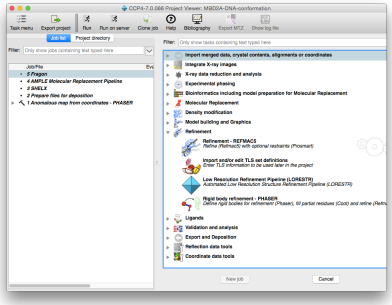


- **Limited sensitivity of most biochemical/ biophysical methods**
- **Fragment-based lead development relies on structural information**
- **Method allows usage of high compound concentration (> 100mM); very sensitive!**
- **History of how to share large-scale infrastructure**
- **Protein Crystallography has seen huge improvements over the last decade:**

Fast detectors

Reliable sample changers

Bright X-ray beams



Advanced software

MAX IV

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

Vetenskapsrådet

SWEDISH RESEARCH COUNCIL

MAX IV

LUND UNIVERSITY

LP3 - Lund Protein Production Platform

SARomics

AstraZeneca

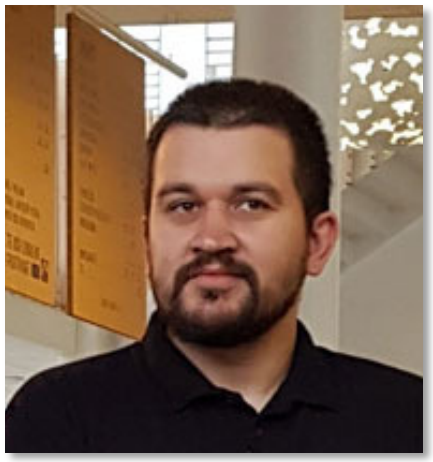
MAX IV

FragMAX team

Overseen by Marjolein Thunnissen (MAX IV Life Science Director)

Tobias Krojer

Sandesh Kanchugal



Elmir Jagudin

FragMAX facility for crystal-based fragment screening

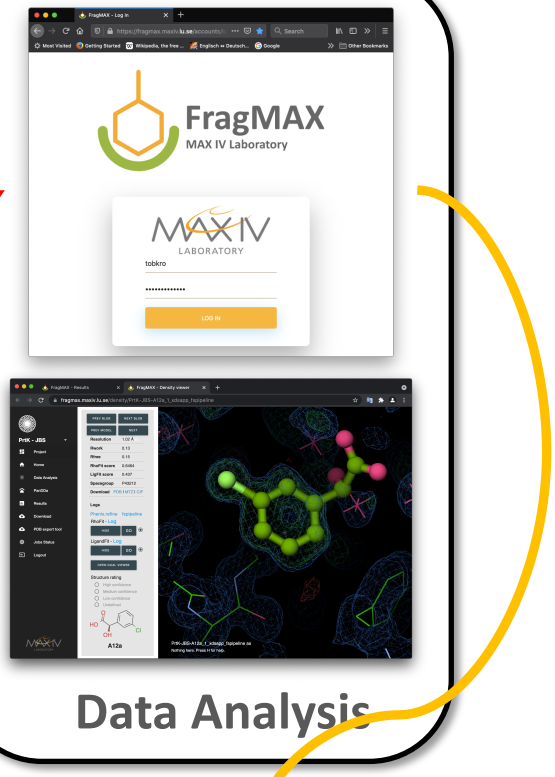
MAX IV Laboratory

https://www.maxiv.lu.se/fragmax

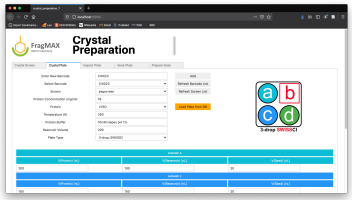
LP3 - Lund Protein Production Platform

Crystal preparation

Data Collection



Data Analysis



Project management software

SQLite database

WORLDWIDE PDB PROTEIN DATA BANK

Lima *et al.* (2020) Acta Cryst D 76, 771–777.
Lima *et al.* (2021) Acta Cryst D 77, 799-808.

Vetenskapsrådet

MAX IV

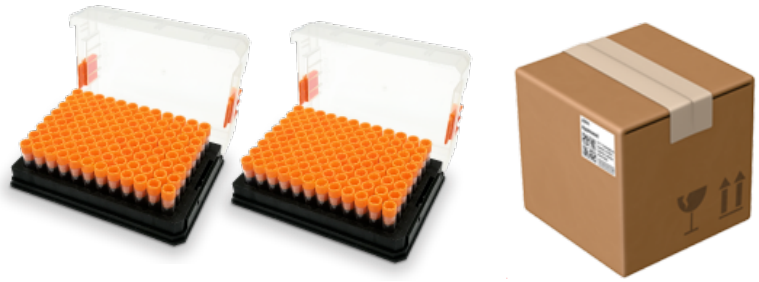
Fragment libraries

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

FragMAXlib

- 170 compounds
- DMSO, EG, powder

Vladimir Talibov



Shipment possible!

EU-OPENSCREEN fragment library

- ca. 1000 compounds + 80 MiniFrags
- In DMSO

Coming soon:

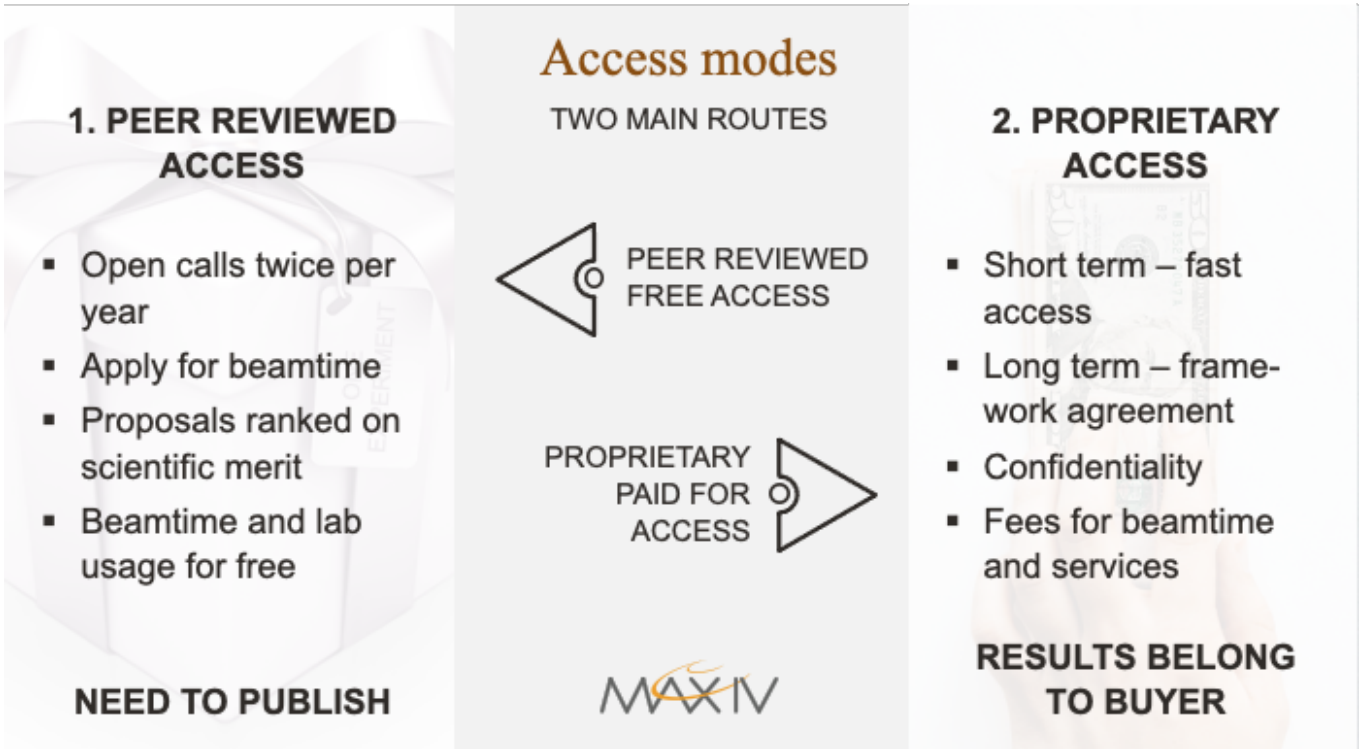
- ENAMINE Essential Fragments (320 cpds) - *tbc*

Upon request:

- F2X entry, FragXtal Screen

Access modes

https://www.maxiv.lu.se/users/proposal-calls



NEW: FAST ACCESS

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

iNEXT DISCOVERY

MAX IV

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 and uniformly diffract < 2Å (ideally); < 2.8Å (minimally)
- Crystal soaking & mounting at FragMAX is done at room temperature
- Crystals need to be tolerant to DMSO (or Ethylene glycol)
- Screening campaign involves screening of approx. 250 compounds

Crystal survey document

FragMAX Crystal Survey

Successful crystallographic fragment screening ultimately depends on the characteristics of the used crystal system. Reliable and reproducible preparation of identical, well-diffracting and easy to handle crystals is the corner stone of the experiment.

Typically, we would assume that users send the protein to MAX IV and that FragMAX staff will set up crystal plates, since it will in most cases not be practical for users to bring crystal plates on site. It is however possible for you to bring your own crystals plates, and it is also possible for you to do the entire crystal preparation, including soaking of fragments, in your home lab. We can send you a copy of our own FragMAXlib library if necessary.

Please fill in the survey below so that we get an idea of the characteristics of your crystal system. This will help us to come up with an optimal strategy for your project and it will also allow us to provide further advice if some properties need further optimization.

General

Where do you plan to perform the experiments?

Crystallization details

Does the protein crystallize readily after thawing? If no, specify required treatment

How many 50 µL aliquots of the protein can you provide?

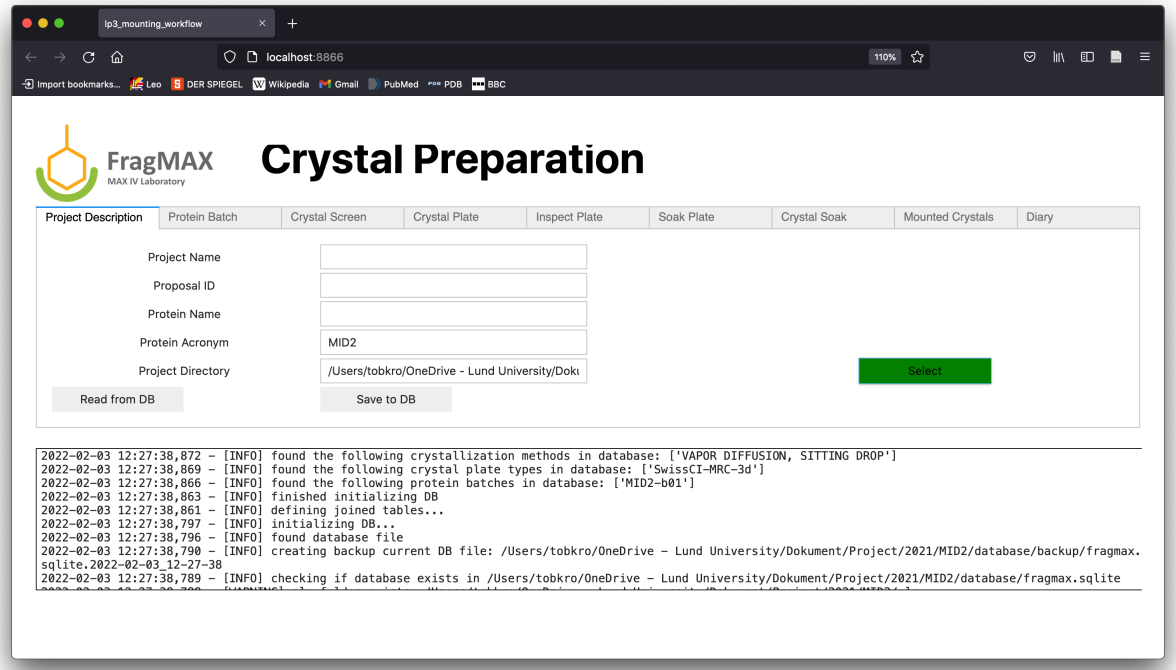
Crystallization condition:

Is seeding required? if yes, specify method

Crystallization temperature: °C

If the crystals do not grow at ambient temperature, do the tolerate to be moved to room temperature?

Example: academic user project (Nov 2021)



Project management software:

- Guides users/ staff through crystal preparation process
- Creates input files for machines and read their output
- Stores all metadata from crystallization to mounted crystal
- Rudimentary electronic lab notebook functionality

day 0 → day 1 → day 2 → day 3 → day 4 → day 5

Example: academic user project (Nov 2021)

day 0: sample shipment & protein crystallization

- (frozen) protein
- Crystallization solution
- (crystal seeds)

SWISSCI

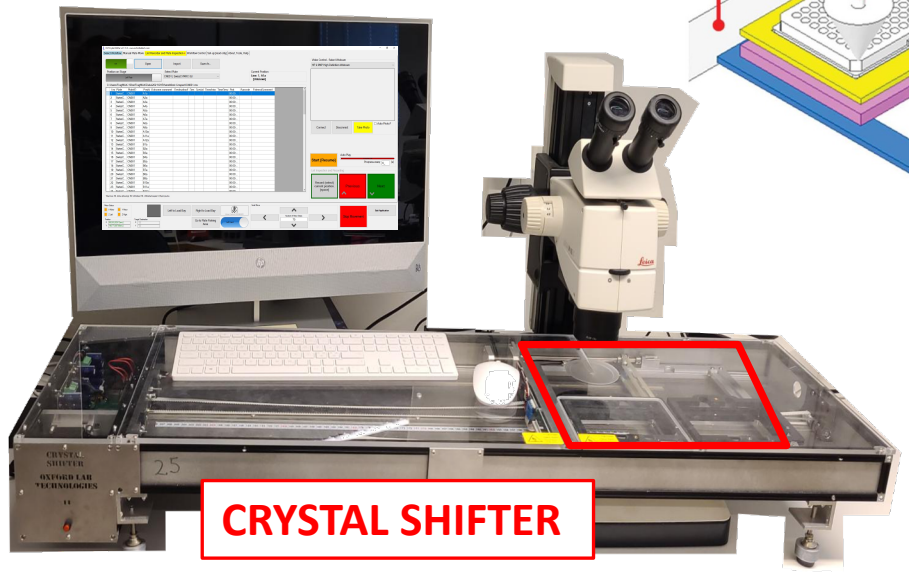
Drop volume:
100 – 500 nL

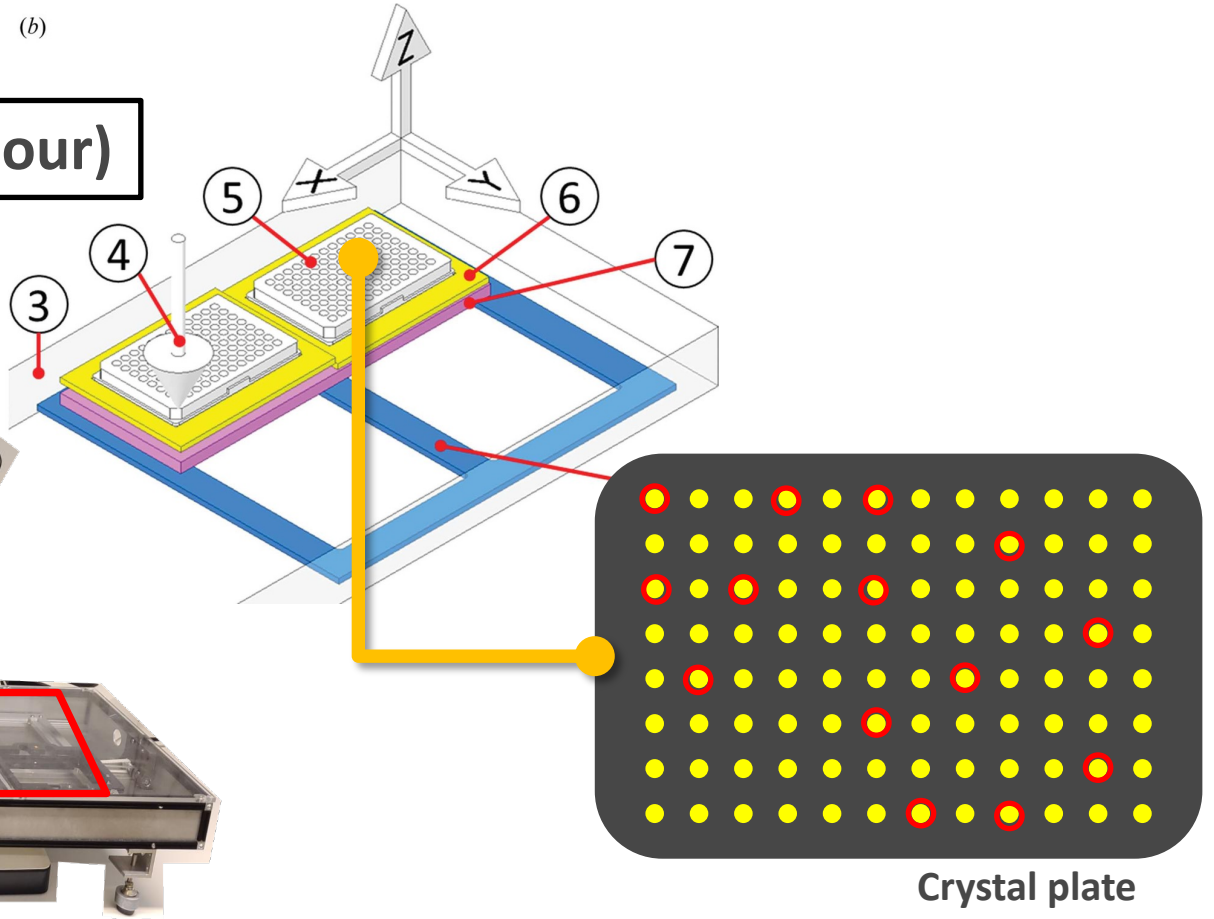
day 0 → day 1 → day 2 → day 3 → day 4 → day 5

Example: academic user project (Nov 2021)

day 1: crystal soaking

1. Crystal selection (~ 1 hour)





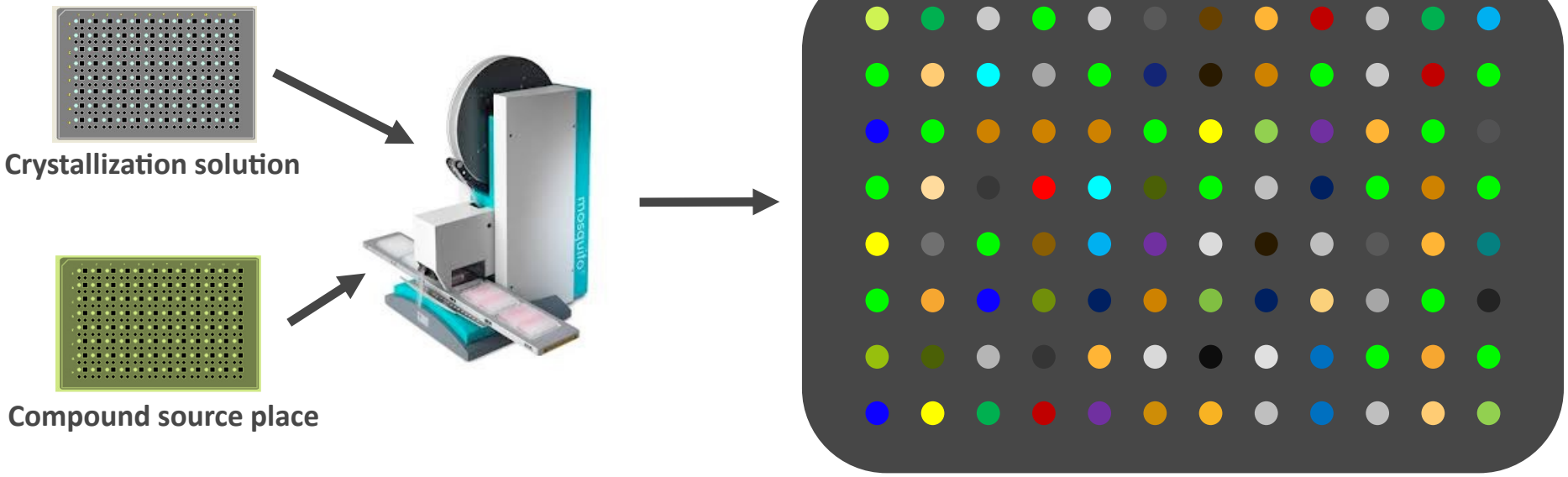
day 0 | **day 1** | day 2 | day 3 | day 4 | day 5

Wright *et al.*
Volume 77 | Part 1 | January 2021 | Pages 62–74 | 10.11107/S2059798320014114

Example: academic user project (Nov 2021)

day 1: crystal soaking

2. Compound plate preparation (~ 30 minutes)

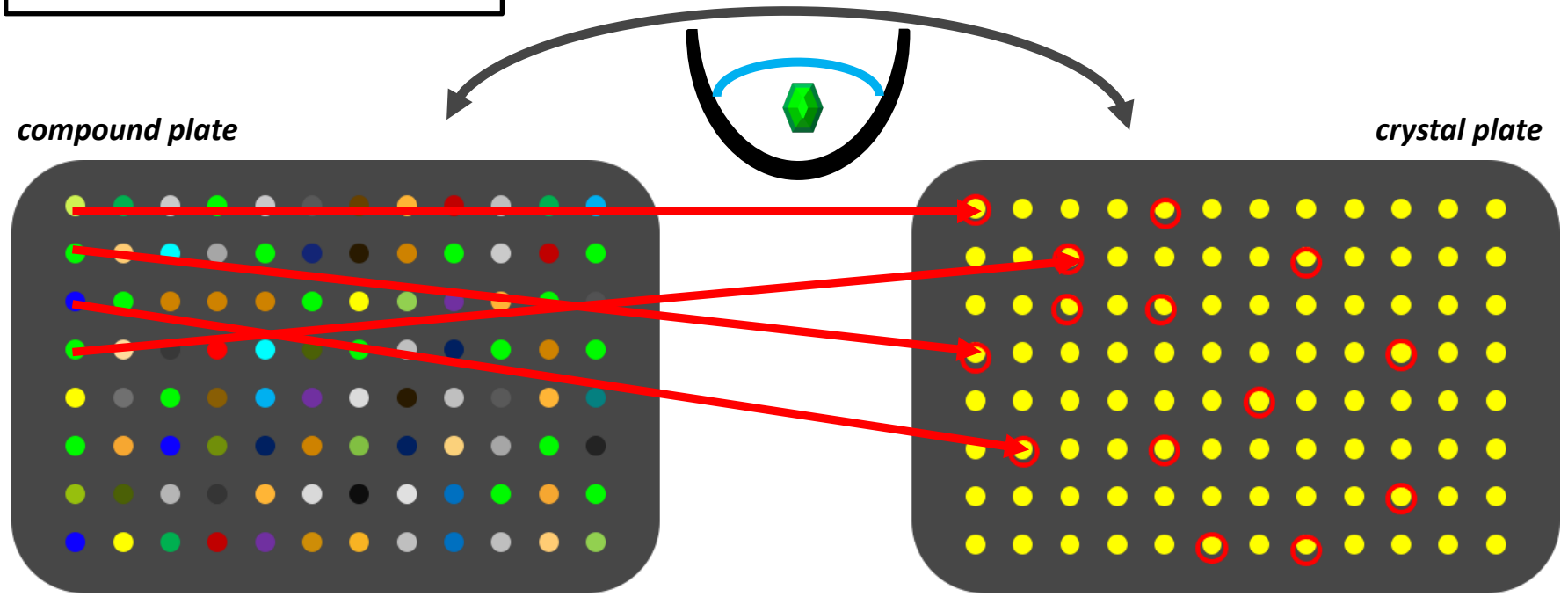


day 0 | **day 1** | day 2 | day 3 | day 4 | day 5

Example: academic user project (Nov 2021)

day 1: crystal soaking

3. Crystal soaking
(1 hour – 170 wells)

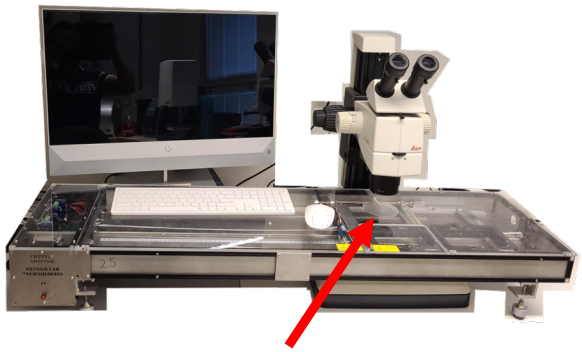


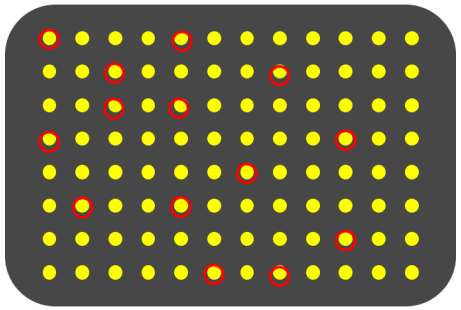
day 0 | **day 1** | day 2 | day 3 | day 4 | day 5

Example: academic user project (Nov 2021)

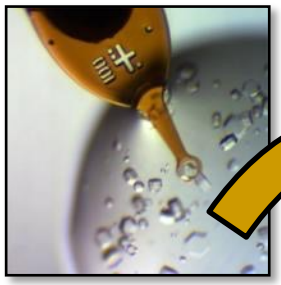
day 2: crystal mounting

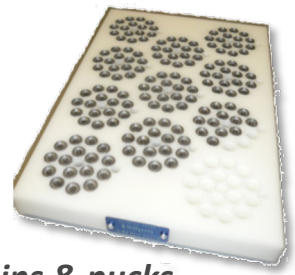
(170 wells & 2 crystals per well -> 340 crystals: 4 hours)





(soaked) crystal plate





pins & pucks

ExiMX

Crystal information for ISPyB database

frozen crystals

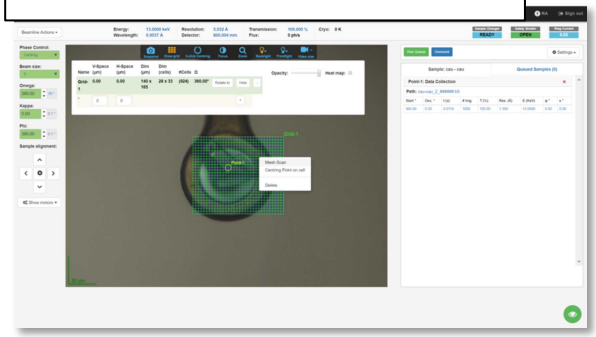
day 0 | day 1 | **day 2** | day 3 | day 4 | day 5

Example: academic user project (Nov 2021)

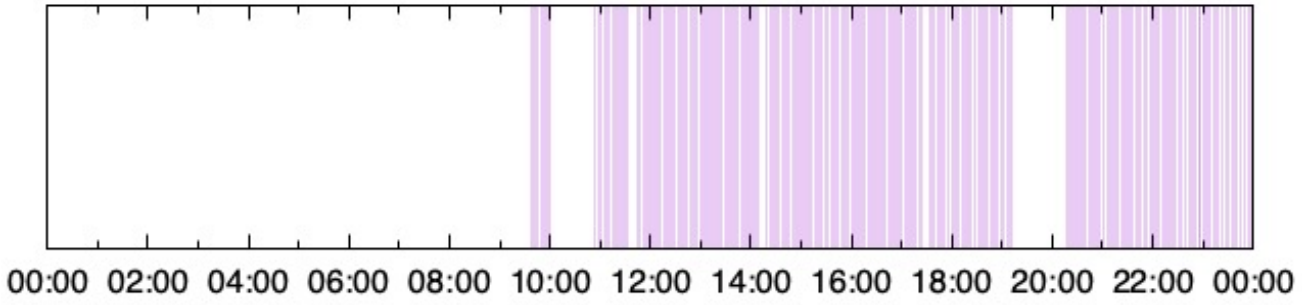
day 3 & 4: diffraction data collection

ISARA sample changer

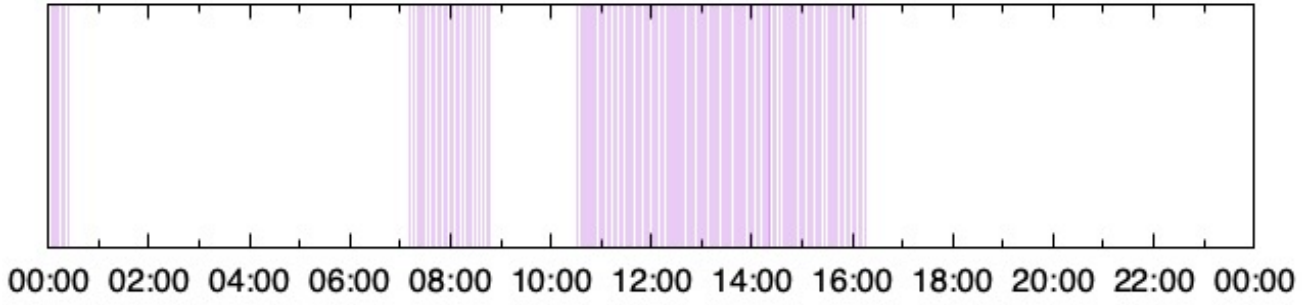
MXCuBE3 user interface



day 1: 255 datasets



day 2: 137 datasets

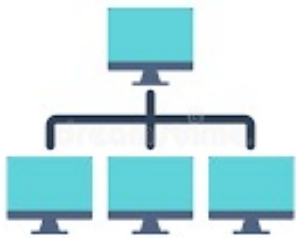


day 0 → day 1 → day 2 → **day 3** → **day 4** → day 5

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

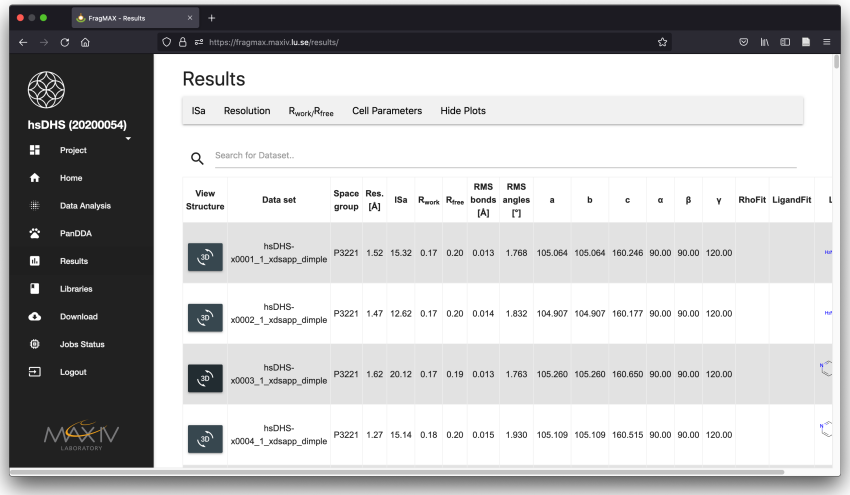
Example: academic user project (Nov 2021)

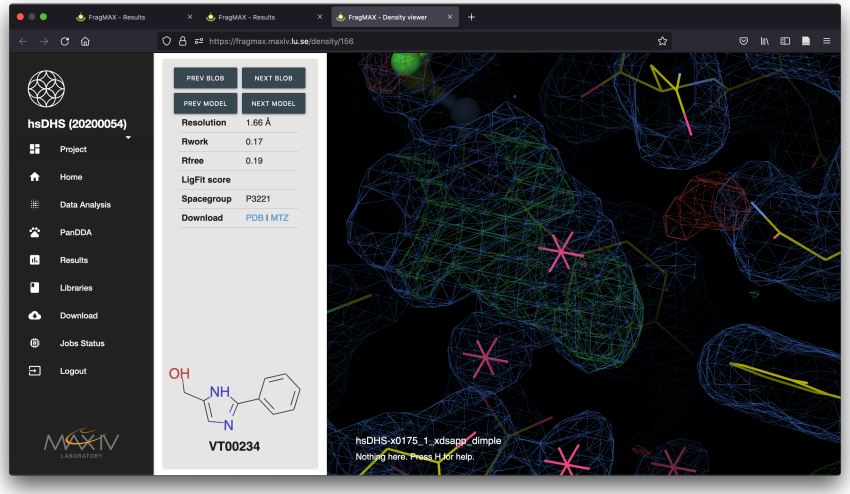
day 5: data analysis



MAX IV computer cluster

https://fragmax.maxiv.lu.se

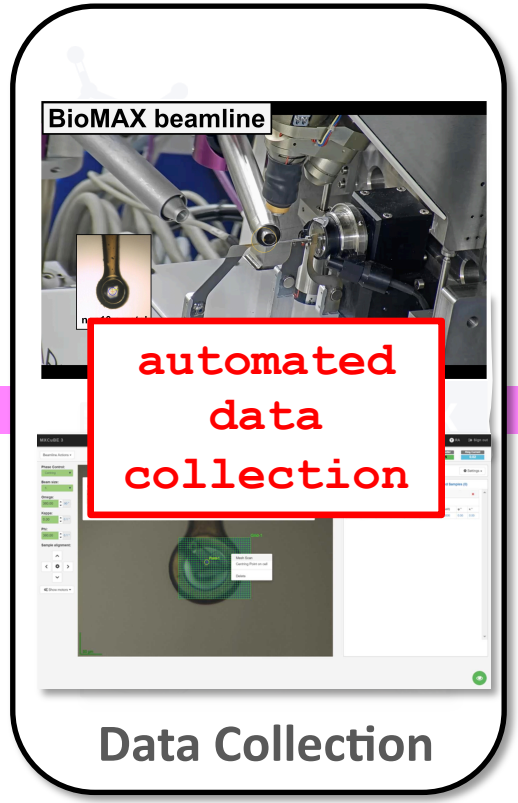


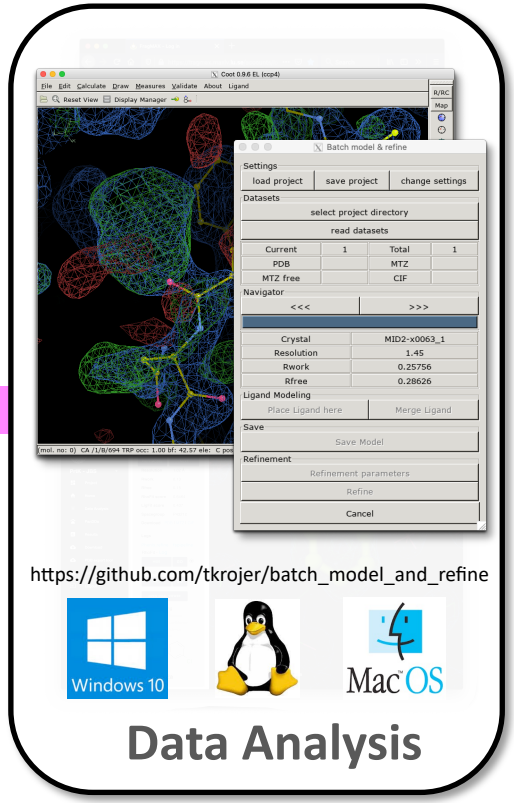


data reduction → **initial refinement**

day 0 → day 1 → day 2 → day 3 → day 4 → **day 5**

Planned improvements for 2022





- Further automation during crystal preparation & data collection
- Bridging the MAP-to-MODEL gap

Future plans

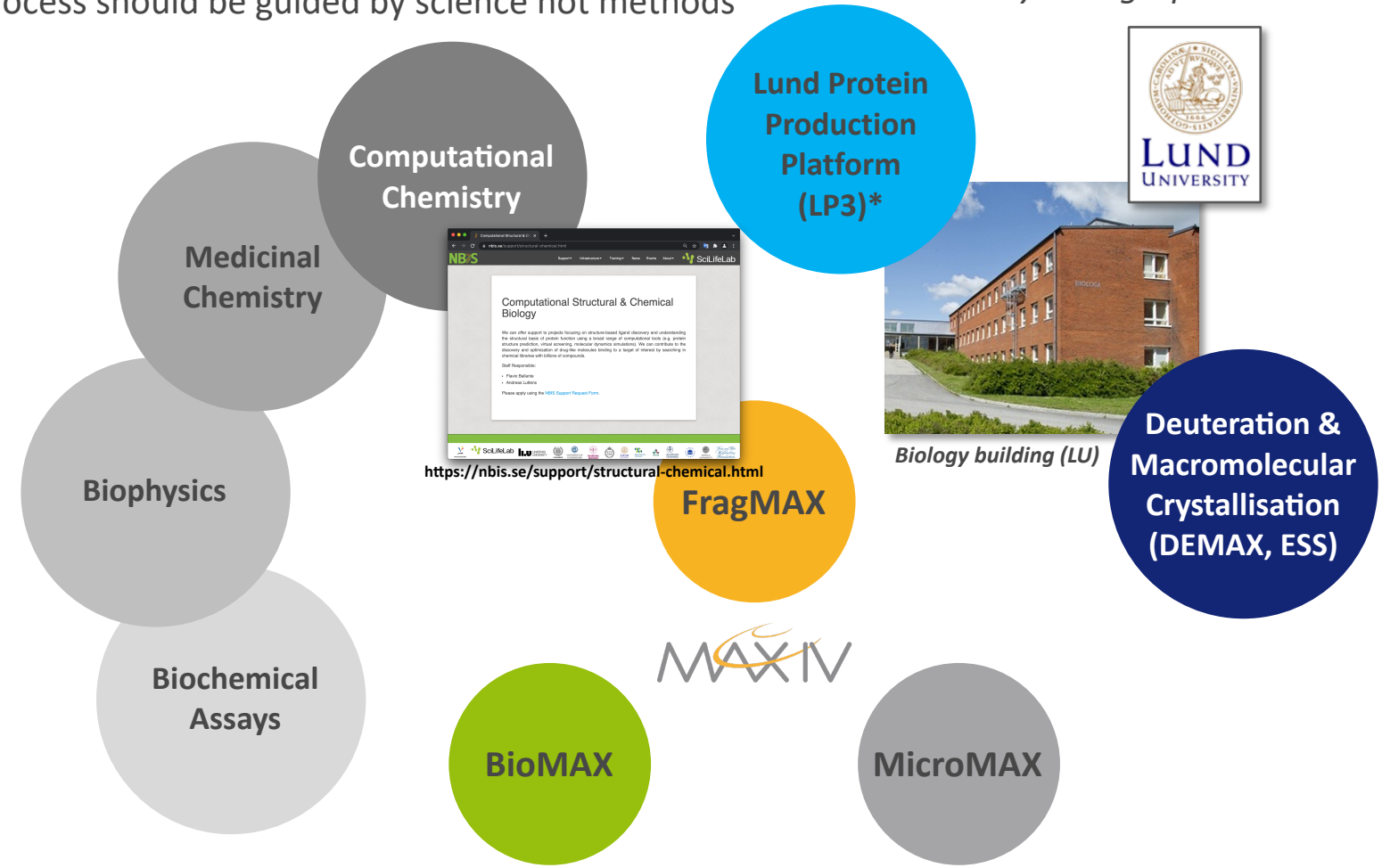
Establishing FragMAX in the Swedish research landscape

The fragment-to-lead challenge:

- Early-state drug discovery is a multidiscipliary effort
- Process should be guided by science not methods

Lund University & FragMAX

- From clone to structure under one roof
- *"Making structural biology accessible to non-crystallographers"*



*) LP3 is now part of Protein Production Sweden (PPS)

Beyond fragment screening

- FragMAX is a *generic platform* for protein-ligand crystal preparation & analysis!
- Protein crystallography undergoes seismic changes: challenges & opportunities
- Connect with other facilities?
- New access modes?

Contact:
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