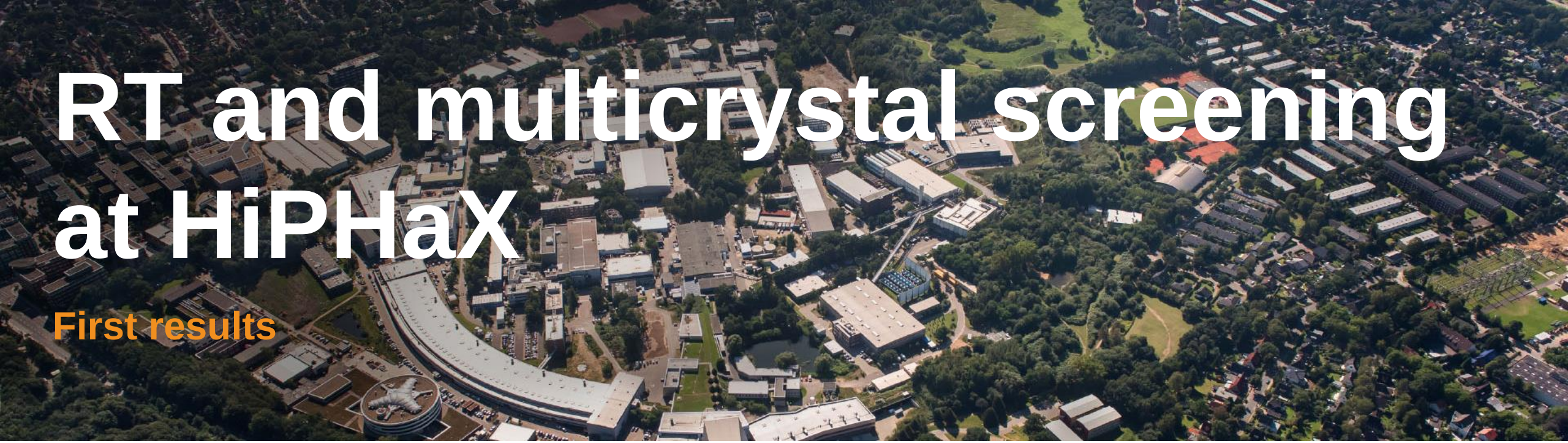


RT and multicrystal screening at HiPHaX

First results

Sebastian Günther
Group FS-BMX

HELMHOLTZ RESEARCH FOR GRAND CHALLENGES

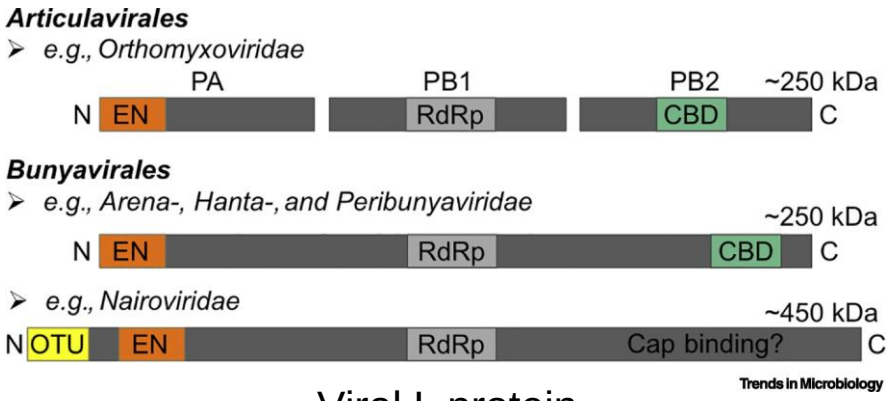
DESY.

Fragment-screening with Cap-snatching endonulceases

First HiPhaX target

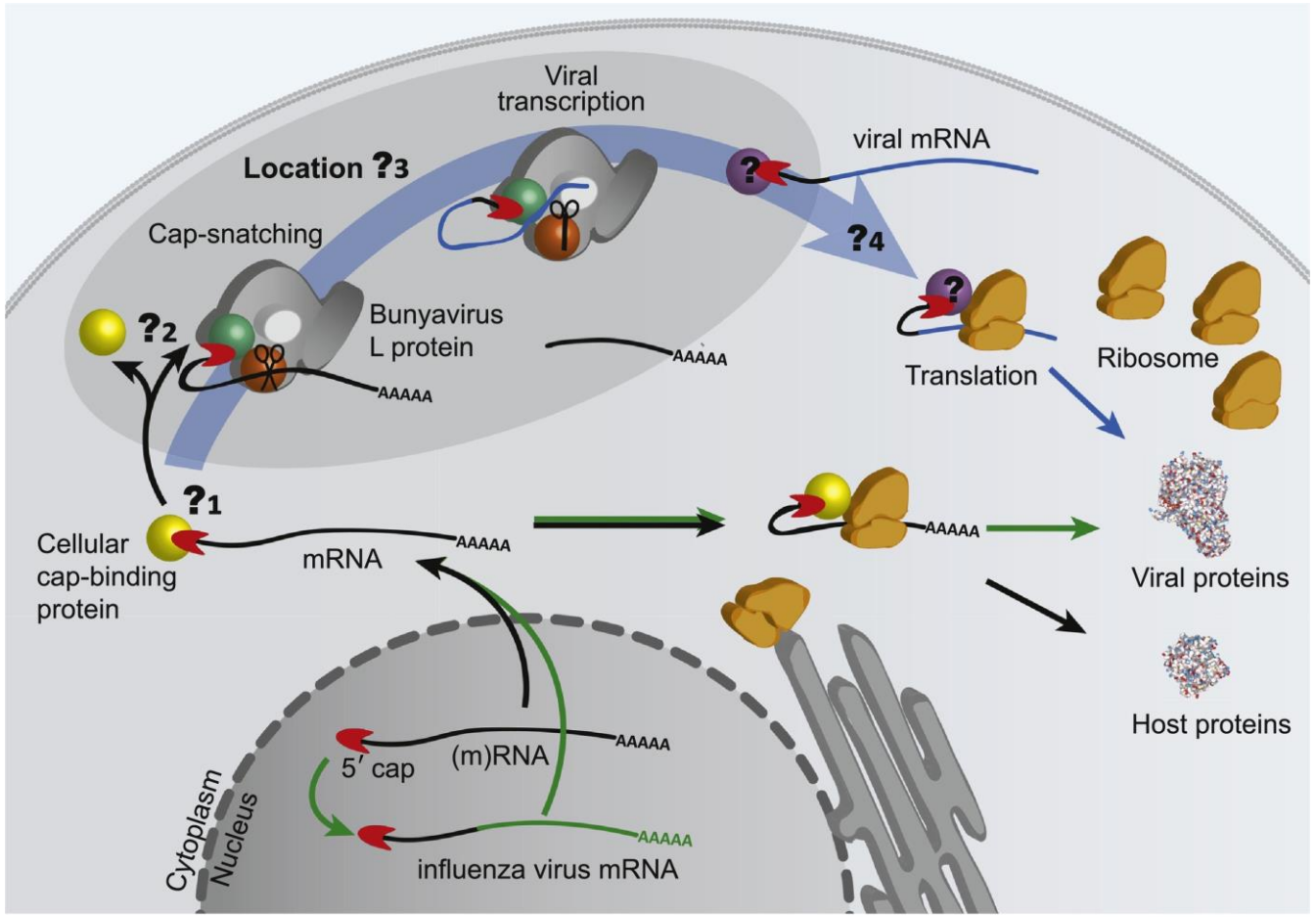
- Develop broad spectrum antivirals against Bunyavirales
  - E.g. Lassa virus, Rift valley fever virus, and Crimean Congo Hemorrhagic fever virus (WHO R&D Blueprint list of priority diseases)

- Target: Cap-snatching endonuclease



Viral L protein

Cap-snatching



Trends in Microbiology 2020 28293-303DOI: (10.1016/j.tim.2019.12.006)

Trends in Microbiology

First room temperature screening at HiPhaX

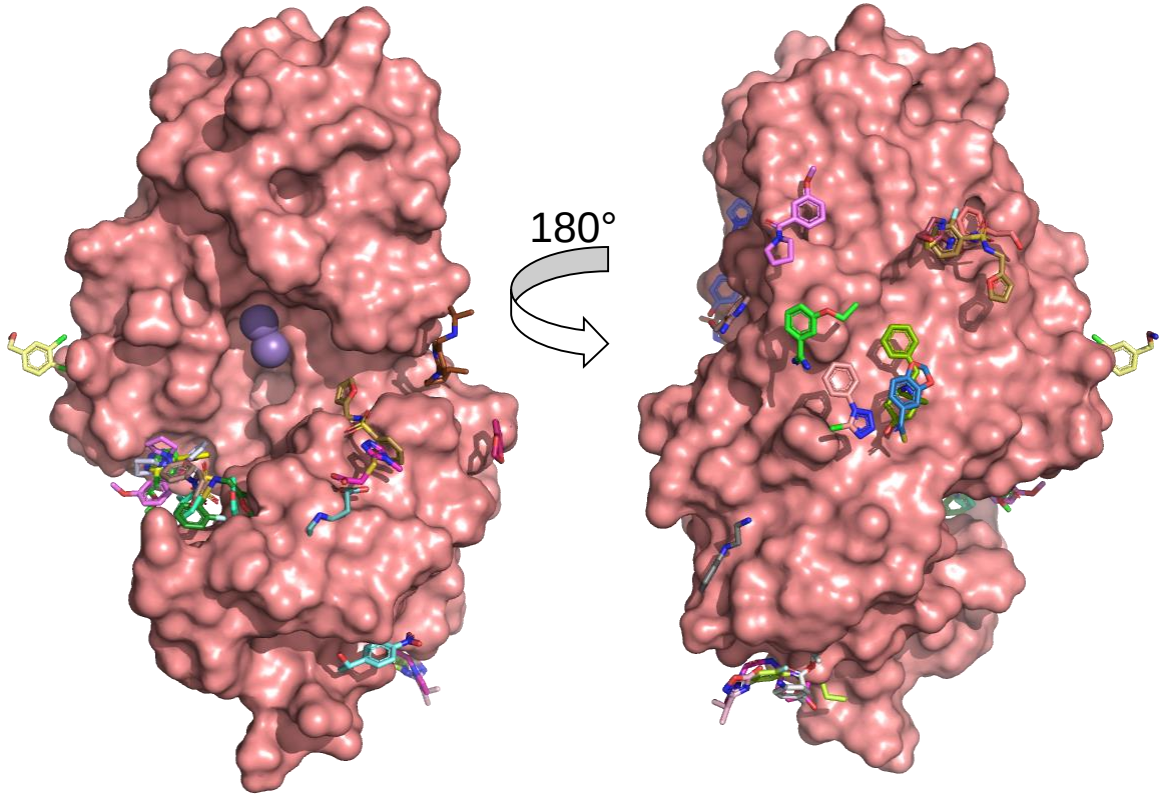
Preliminary results

➔ use HTS do define common interactions in binding site

➔ basis for development of pan-Bunyavirales inhibitors

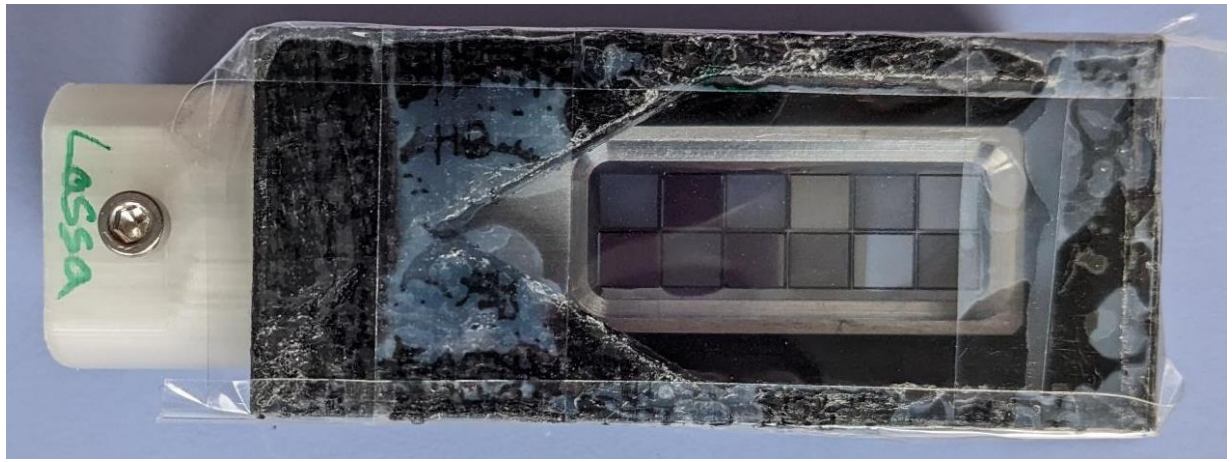
- Cap-snatching endonuclease of *Bunyaviridae* L-Protein from Lassa virus (LASV)
- F2X-Entry Screen (96 fragments)
- Co-crystallization with fragments
- Single crystals with sleeve
- 325 data sets collected within one day

LASV Cap-EndoA



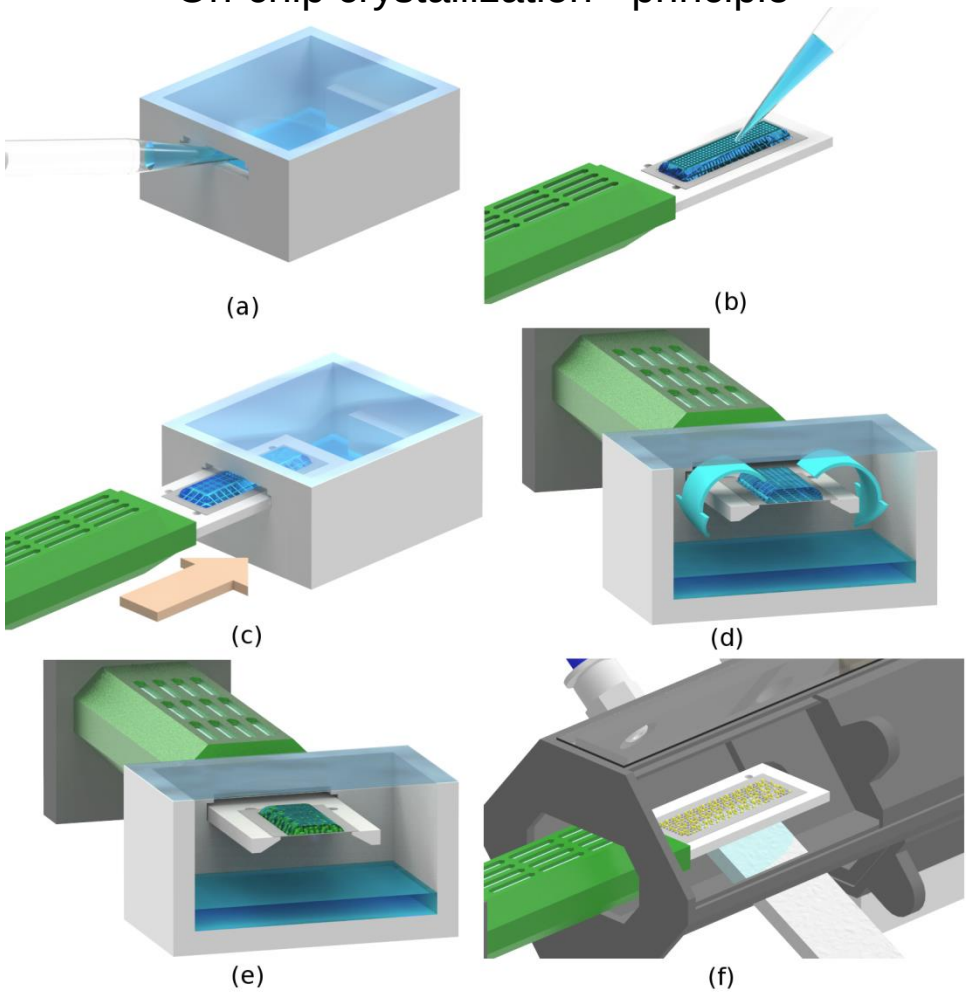
Hits identified by PanDDA
Hit rate 26%

First multicrystal data collection



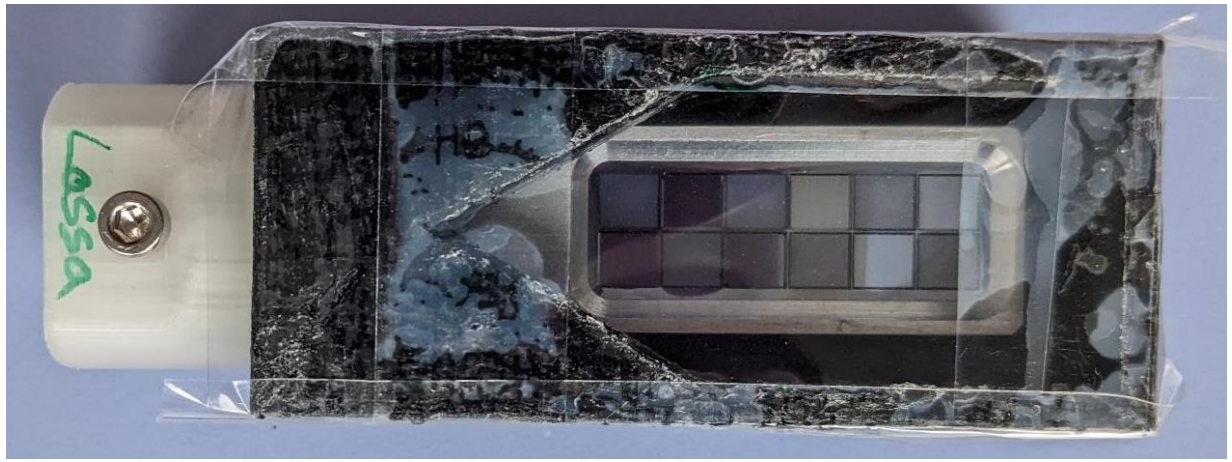
On-chip crystallization of protein
Followed by soaking with compounds
1 compound/well

On-chip crystallization - principle



Lieske, J. et al. (2019). IUCrJ 6, 714-728.

First multicrystal data collection

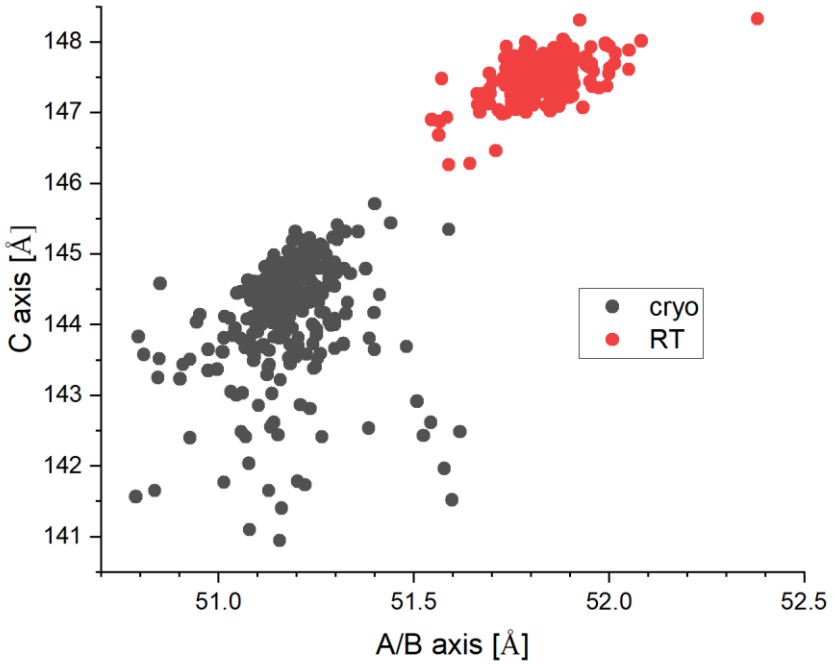


On-chip crystallization of protein
Followed by soaking with compounds
1 compound/well,
Data collection in serial crystallography mode

New design of chip holder and crystallization chamber

Variability cryo vs. RT

Unit cells LASV screen
space group $P4_12_12$



RT and controlled humidity
decreases the unit cell variations

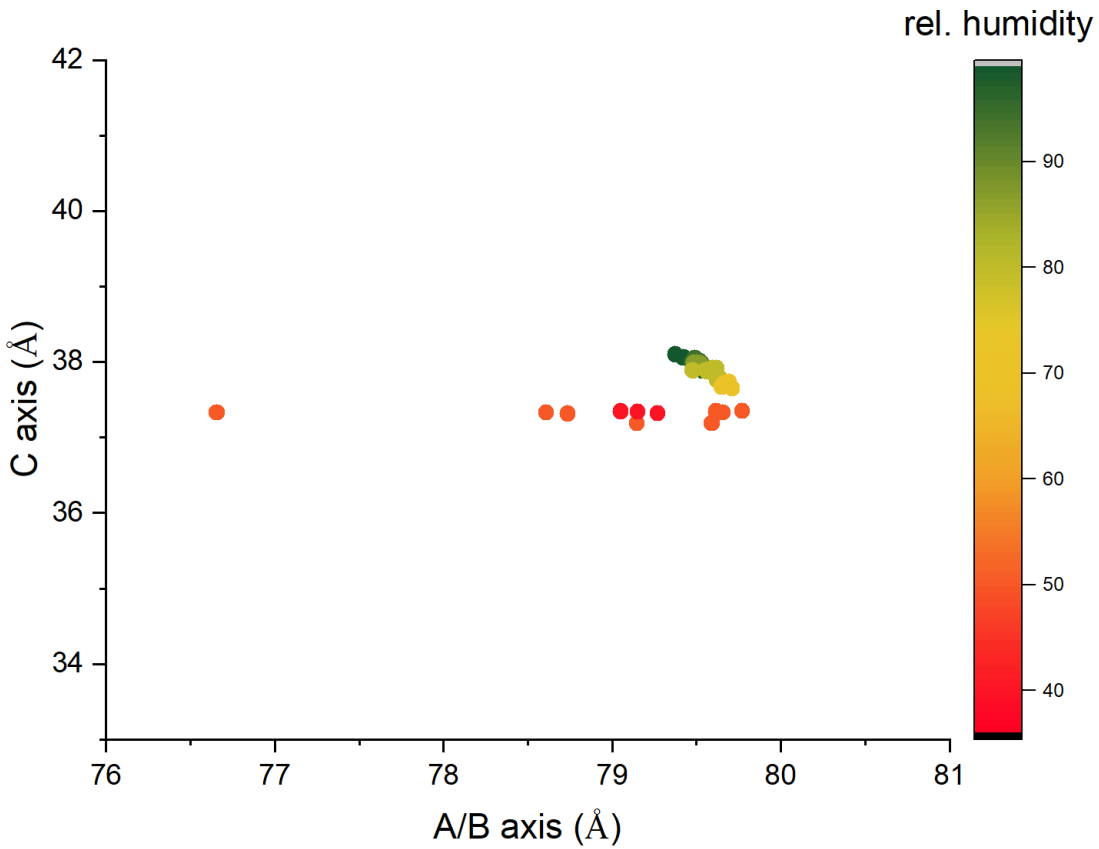
Changing humidity ➔ structural variability

- (Uncontrolled) variability hinders hit finding
- Controlled changes can help define structural variability
- Better apo models for dataset clustering

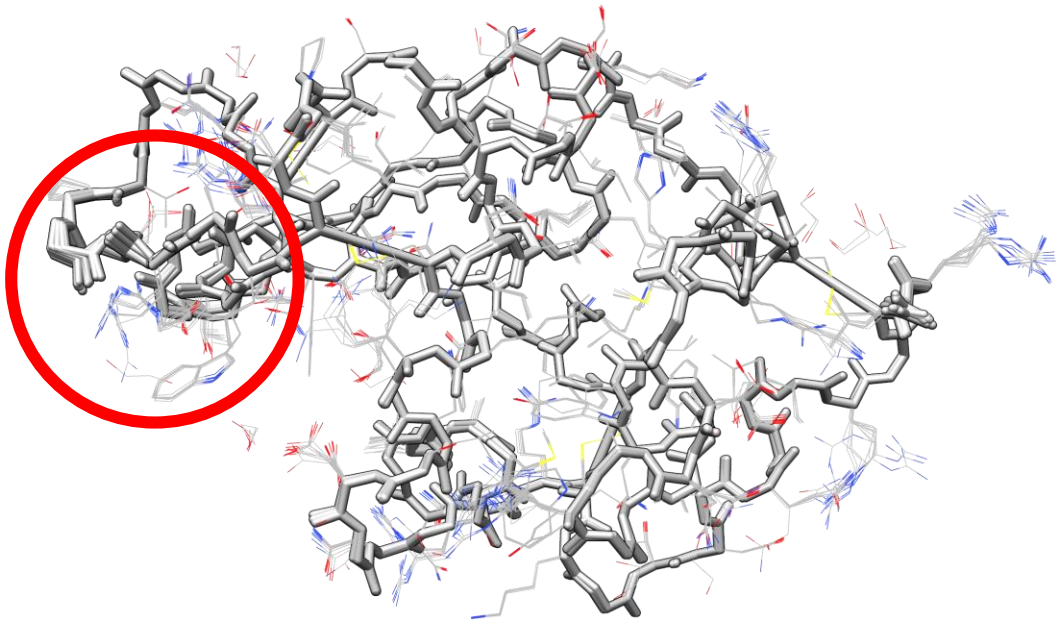
Humidity enforces structural variability

Lysozyme

cell axis changes

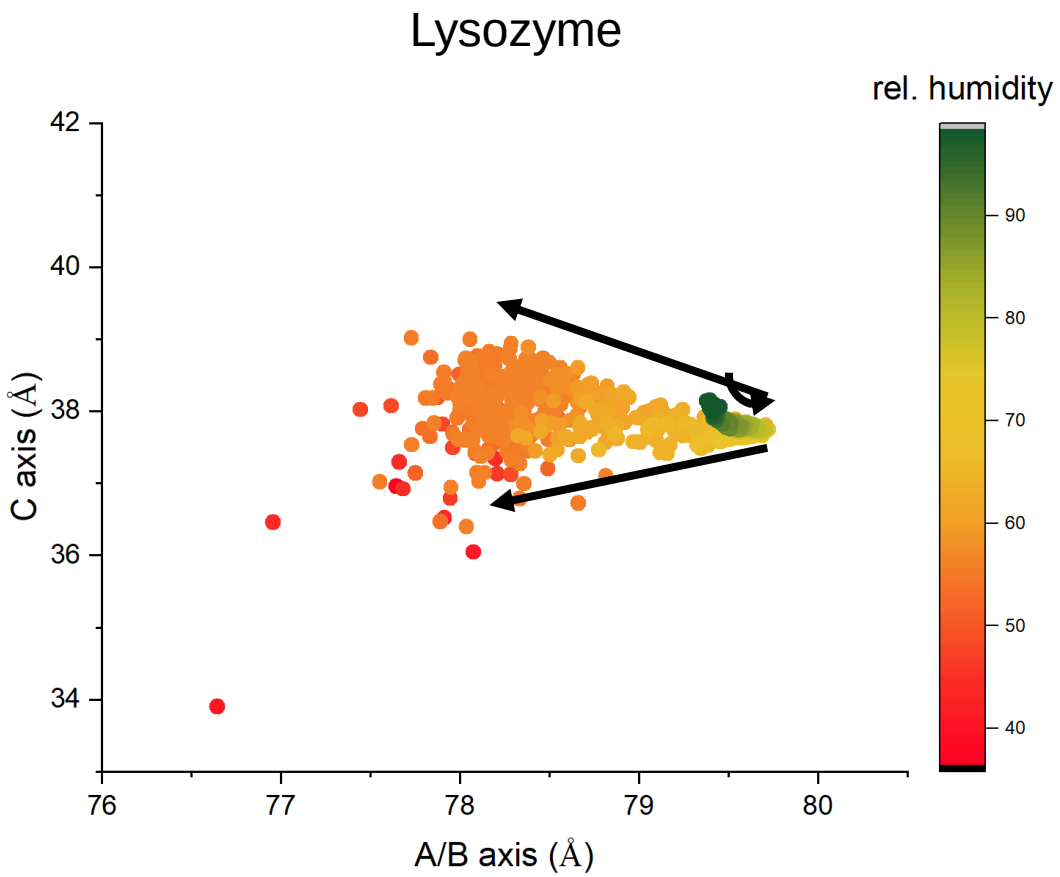


Structural changes

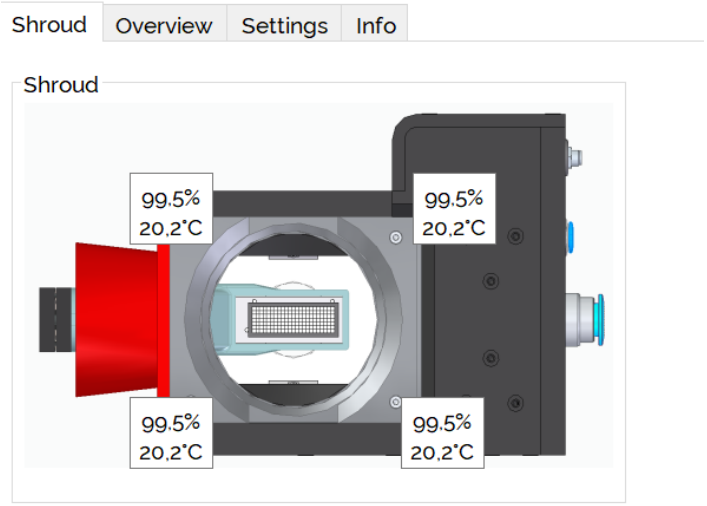


Power of serial crystallography

Continuous analysis of humidity induced changes

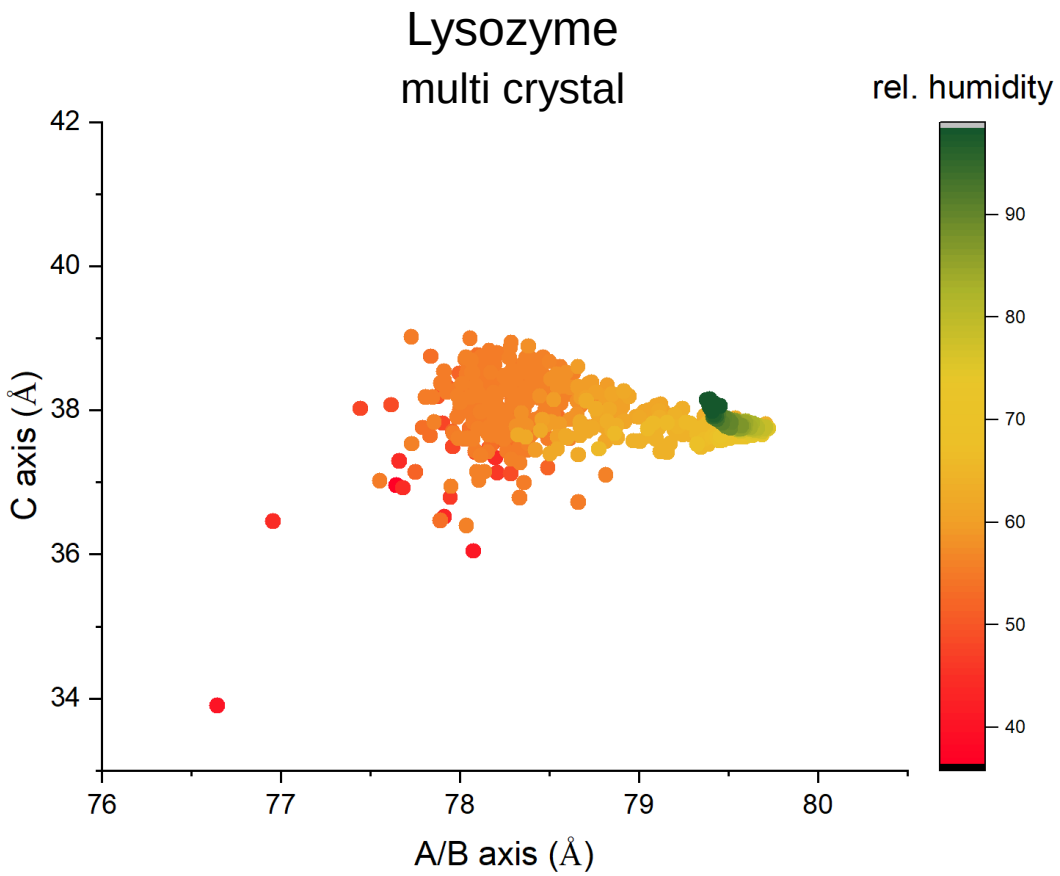


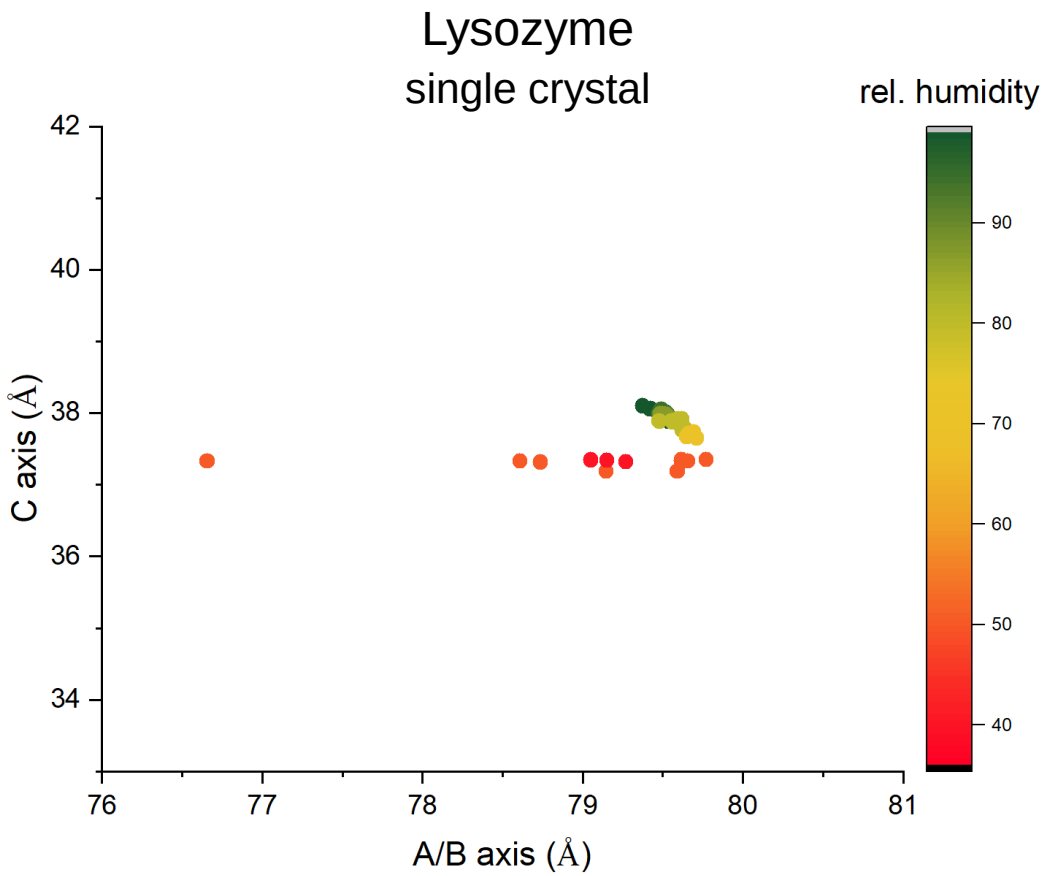
- Lysozyme crystals on chip
  - 2 µL of dense crystal slurry
- Continuous change of rel. humidity from 98 to 36 %
- (semi)-serial data collection
  - 45 deg wedges
  - >1800 scan points/about 900 with useful data



Power of serial crystallography

Continuous analysis of humidity induced changes





Power of serial crystallography

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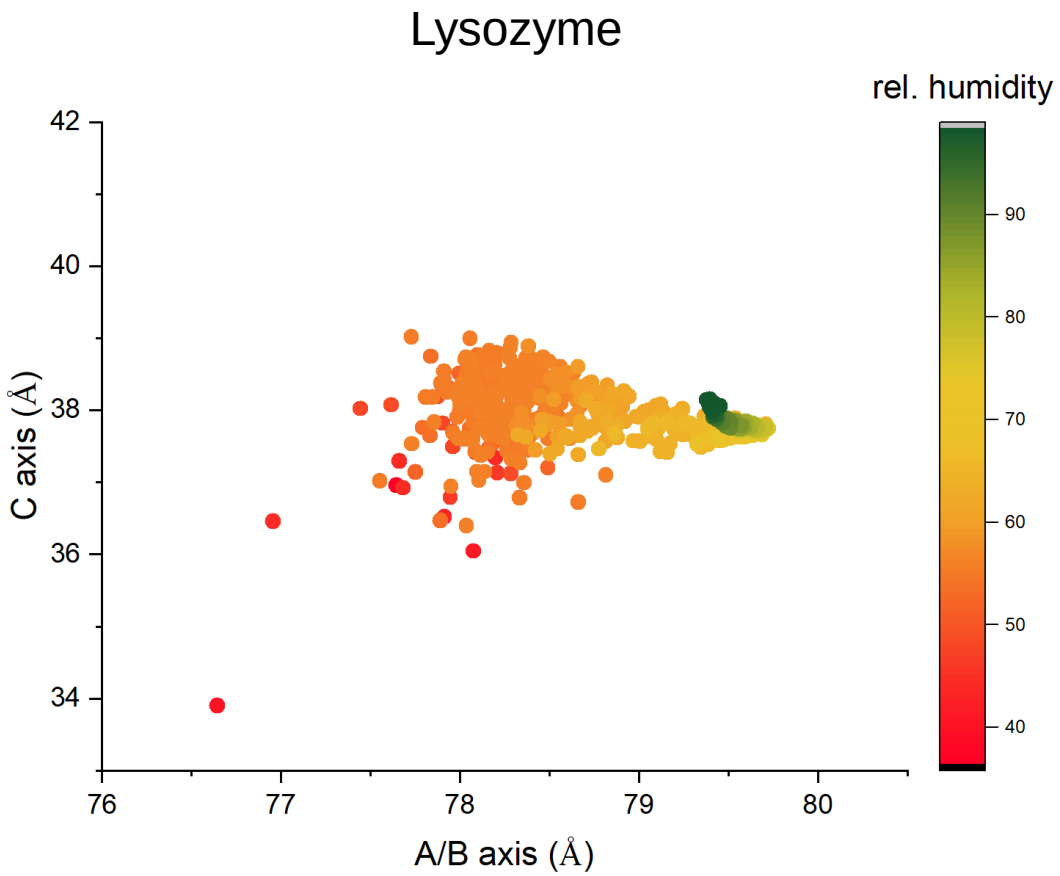

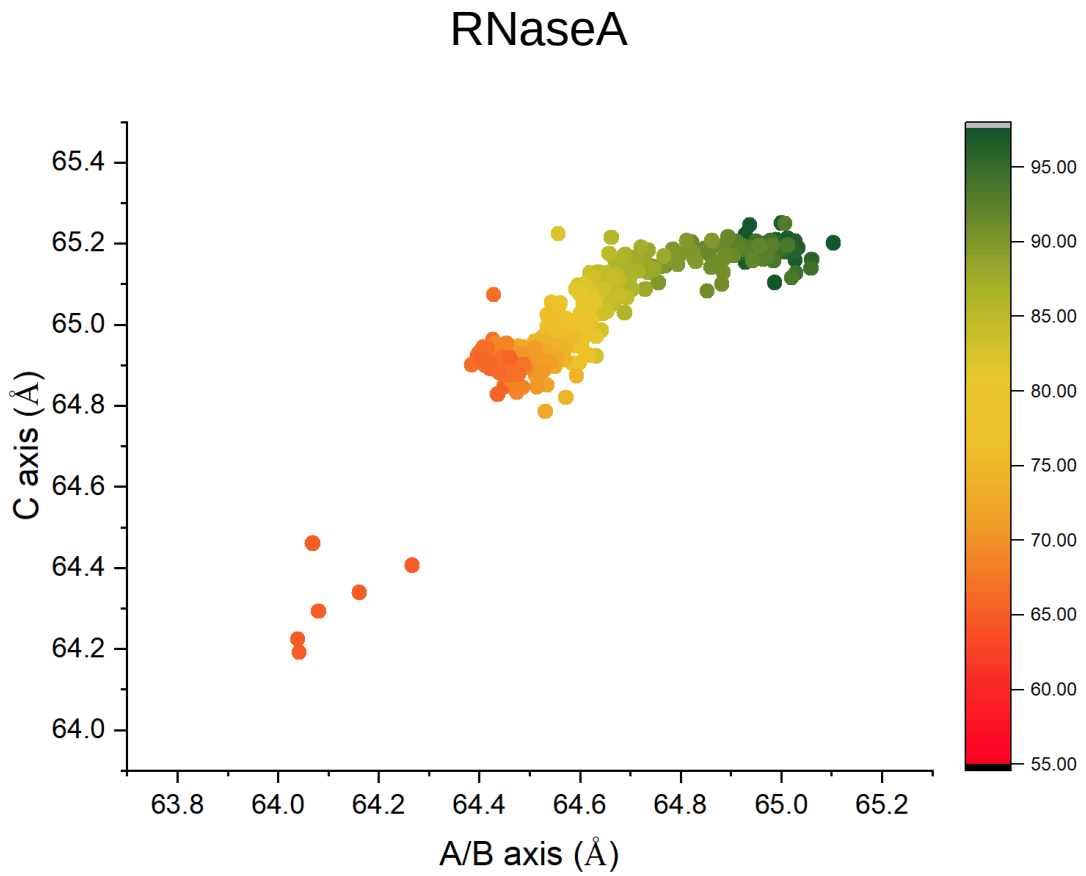

Different behavior of different crystal systems

Summary

- First RT screen at HiPhaX
  - Fragments targeted against LASV Cap-Endo
- Next approach: serial wedge data collection to improve data quality
- Revealing protein flexibility through finely tuning humidity (and temperature) to induce changes in protein structure
  - Conformational changes in lysozyme
  - Systematic changes in apo references datasets to increase quality of hit identification by PanDDA or SBD

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Thank you