Crystallographic Fragment Screening: Identification of promising molecules binding the SARS-CoV-2 PlPro protease

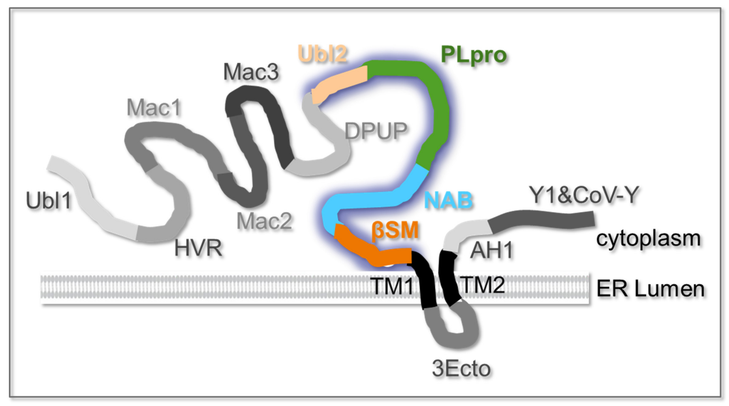
G.L. Freiherr von Scholley[2], M. S. Lopez[2], R.C. Hillig[1], C. Mueller-Dieckmann[2], E. Kandiah[2], M. Schäfer[1]

[1] NUVISAN ICB GmbH, Berlin, GERMANY [2] ESRF, Grenoble, FRANCE

Introcuction

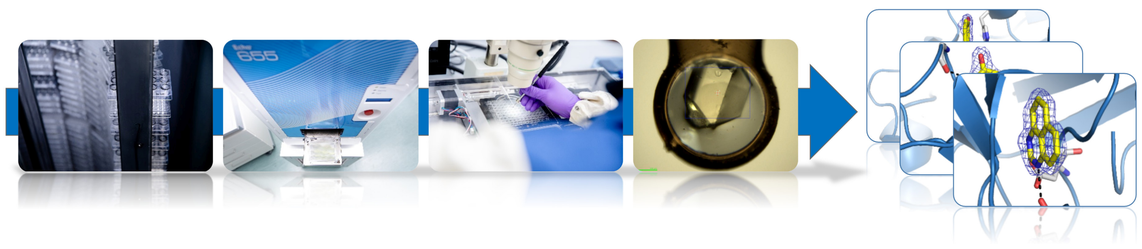
The Covid-19 pandemic has been successfully tackled by the quick development of mRNA vaccines. While vaccines are very effective in slowing the viral spreading, therapeutic drugs help deal with severe cases of infection. So far, only one drug has been developed against the SARS-CoV-2 Mpro. To combat the ever-increasing number of SARS-CoV-2 variants and prepare for future coronavirus outbreaks, we addressed the Papain-like Protease (PlPro) domain of the non-structural protein 3 (NSP3), which plays a crucial role in viral replication.

We successfully performed a crystallographic fragment screen (HTX) on the target domain Ubl2-PlPro, measuring ~800 different fragments of Nuvisan's X-ray fragment library that resulted in multiple promising hits.



Schematic representation of SARS-CoV2 NSP3; NSP3 is the largest polyprotein precursor and responsible for cleavage of viral proteins. It is involved in vesicles formation crucial for replication and in innate immune system suppression

HTX Workflow

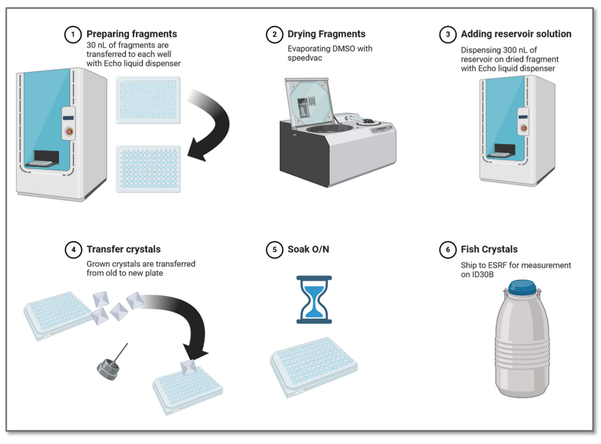


Overview of integrated HTX workflow:

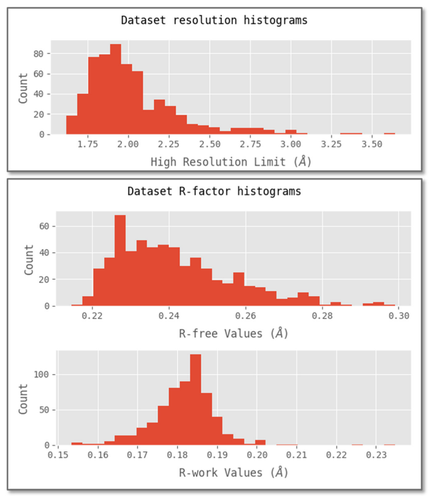
- **Automated imaging systems** at 4°C and 20°C for plate inspection.
- **Echo Acoustic Liquid Handler** for soaking large number of HTX fragments into crystals.
- **Crystal Shifter** used for freezing crystals for data collection.
- Visual Basic scripts allow for **reliable data tracking** throughout the complete workflow.
- **Fast and reliable crystallographic data collection** through continuous access to high-brilliance synchrotron sources.
- In-house **proprietary data analysis pipeline**, including **PanDDA**, enables quick identification of datasets with a high likelihood of fragment binding.

HTX Fragment Screen

- Ubl2-Pro^{C111S} mutant construct yielded crystals suitable for HTX
- Crystals dissolved upon soaking with fragments in DMSO
  → **DMSO free soaking protocol** established
- **HTX screen** performed using **Nuvisan's proprietary fragment library**
- **Automated data collection** at beamline ID30B at ESRF: **1022 datasets**
  - 763 datasets processed using autoPROC staraniso
  - All datasets were analyzed using PanDDA
  - 256 potential PanDDA hits inspected manually
  - **56 hits** identified and refined with Refmac



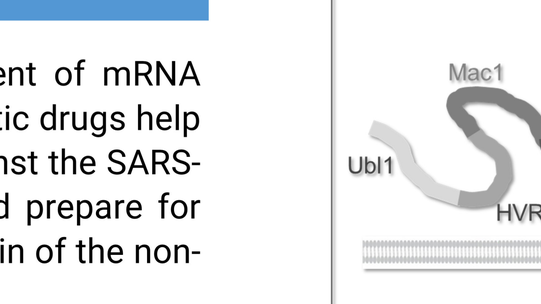
Dry soaking protocol for Ubl2-Pro^{C111S} crystals



PanDDA statistics of automatically collected data sets at ESRF showing good quality of data

Results

- Ubl2-PlPro^{C111S} crystallizes as dimer in SG $P\,2_12_12$.
- Mainly two different binding sites
  - **Site 1** is close to active site → **10 fragments bound**
  - **Site 2** located in thumb domain next to Ubl2 → **8 fragments bound**
- Site 2 only present in chain B of Ubl2-PlPro^{C111S} dimer
- **Additional site** identified
- Fragment hits do not bind to both protein chains at one time

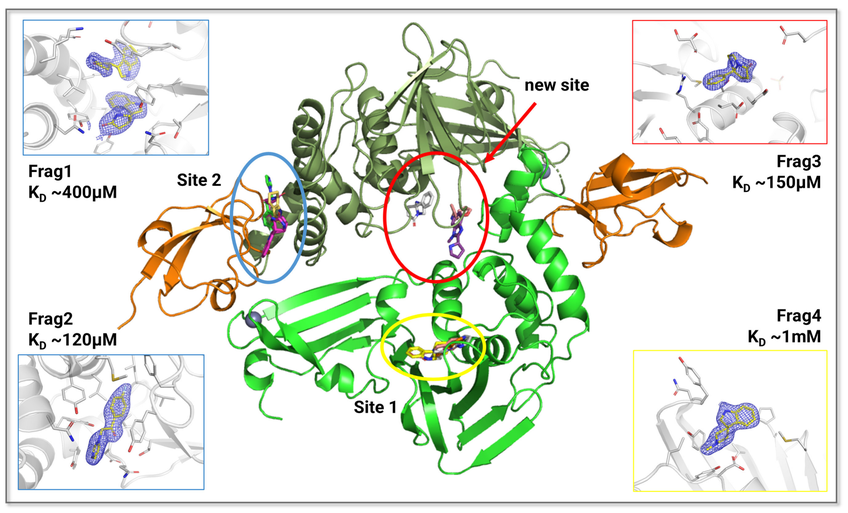


Two main binding sites in Ubl2-PlPro
Site 1 close to active site
Site 2 in thumb domain close to Ubl2

Chain A and Chain B in Ubl2-PlPro differ with RMSD of 0.3 Å

SPR Analysis

- 16 fragments were re-ordered for biophysical analysis of which **14 fragments were confirmed** as binders.
- **8 hits** showed K_D values in **low 3-digit µM** range
- Best fragment (Frag2) was bound to Site 2 with a K_D of ~120µM.



Dimer of Ubl2-PlPro showing all 8 fragments as stick models. Electron densities for four fragments showing best K_D values are shown. Frag 1 binds twice in Site 2.

Summary & Conclusion

- **New fragment binders** for important target SARS-CoV-2 PlPro identified by HTX.
- Fragments bind mainly to **two very promising sites**.
- Frag3 binds to a new site with encouraging K_D.
- In the next steps, fragments will be further evaluated using SAR-by-catalogue and biochemical assays.