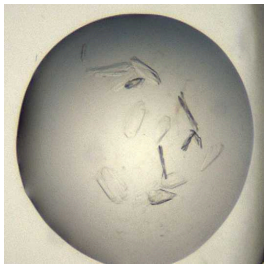
May 13, 2025 Version 2

Crystallisation of SARS-CoV-2 MPro in $P2_12_12_1$ for compound soaking V.2



Version 1 is forked from Crystallization of SARS-CoV-2 Mpro

DOI
dx.doi.org/10.17504/protocols.io.261ge5emyg47/v2

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Blake Balcomb: The principle crystallographer on the SARS-CoV-2 Mpro project.;

ASAP Discovery

Peter Marples
Diamond Light Source

DOI: https://dx.doi.org/10.17504/protocols.io.261ge5emyg47/v2

External link: https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-COV-MPRO

Protocol Citation: Blake Balcomb, Peter Marples, Lizbé Koekemoer, Charlie Tomlinson, Daren Fearon 2025. Crystallisation of SARS-CoV-2 MPro in P212121 for compound soaking. **protocols.io** https://dx.doi.org/10.17504/protocols.io.261ge5emyg47/v2Version created by **Mary-Ann Xavier**

Manuscript citation:
Melissa L. Boby *et al.*,
Open science discovery of potent noncovalent SARS-CoV-2 main protease inhibitors.*Science***382**,eabo7201(2023).DOI:10.1126/science.abo7201

Protocol status: Working
We use this protocol and it's working

Created: February 24, 2025

Last Modified: May 13, 2025

Protocol Integer ID: 123294

Keywords: crystallisation, XChem, ASAP, AViDD, Diamond Light Source, i04-1, Mpro, SARS-CoV-2, crystallisation of sar, p212121 for compound, reproducible sar, crystallization protocol, xchem fragment screening, severe acute respiratory syndrome coronavirus, crystallisation, solvent test, strategies for pandemic preparedness, pandemic preparedness, sar, cov mpro

Funders Acknowledgements:
National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAID)
Grant ID: Grant ID: U19AI171399

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgements:

Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0QX, UK
Research Complex at Harwell, Harwell Science and Innovation Campus, Didcot OX11 0FA, UK
Oxford Lab Technologies crystal shifter https://doi.org/10.1107/S2059798320014114

Abstract

The COVID-19 pandemic has highlighted the need to identify novel therapeutic interventions and strategies for pandemic preparedness against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This protocol outlines the crystallization protocol and buffer conditions used to obtain reproducible SARS-CoV-2 Mpro crystals suitable for **XChem** fragment screening. In this new version we added the solvent test, soaking conditions, seeding reference paper and addgene ids used in this experiment.

Materials

SwissCI 3 lens crystallization plates **https://swissci.com/product/3-lens-crystallisation-plate/ Codes:**
Midi: UVXPO-3LENS 3W96T-PS 3W96T-UVP

[M] 1 Molarity (M) MES adjusted to pH 6.7 with HCl, Molecular Dimensions, Catalog # MD2-013-PH 6.7
50% w/v PEG 4000, Molecular Dimensions, Catalog # MD2-250-11
99.9% DMSO, Molecular Dimensions, Catalog # MD2-250-159

Purified SARS-CoV-2 Mpro protein ([M] 5 mg/mL) in [M] 10 millimolar (mM) HEPES, pH 7.5 ,
[M] 0.5 Molarity (M) NaCl, 5% glycerol, [M] 0.5 millimolar (mM) TCEP

Protocol materials

SARS-CoV-2 isolate: Wuhan-Hu-1 Mpro (3CL protease) **addgene Catalog #**228666

SARS-CoV-2 Mpro protease (C145S mutant - catalytically inactive); aliases: 3CLpro, 3C-like **addgene Catalog #**204769

Troubleshooting

Safety warnings

Follow all handling warning for the chemicals used in the crystalllisation screen composition.

SARS-CoV-2 Mpro expression and purification

1 The protein used for crystallisation was expressed and purified using the following protocol and plasmid

SARS-CoV-2 isolate: Wuhan-Hu-1 Mpro (3CL protease) **addgene Catalog #**228666

Protocol

NAME

SARS-CoV-2 Mpro small scale expression and purification protocol

CREATED BY

Korvus Wang

Preview

Equipment needed

2 **Formulatrix Rock Imager** (or incubator of choice)
SPT mosquito

Equipment

Mosquito HV	NAME
High Volume 16-Channel Robotic Liquid Handler	TYPE
SPT LabTech	BRAND
3097-01057	SKU
https://www.sptlabtech.com/products/liquid-handling/mosquito-hv/	LINK

P100 8 multi-channel pipette

SwissCI 3 lens plate

Crystallisation experiment

1d

3 Preparation of seed stock using imature

SARS-CoV-2 Mpro protease (C145S mutant - catalytically inactive); aliases: 3CLpro, 3C-like **addgene Catalog #**204769

Mpro and mature protein: Protocol for obtaining SARS-CoV-2 Mpro $P2_12_12_1$ crystal form can be found here: DOI: https://doi.org/10.1016/j.jmb.2021.167118.

4 **Prepare seed stock:** 17m 40s

Protocol

NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

Preview

1: 250 dilution Sample seeds

5 **Protein and buffer requirements:**

43.2 µL [M] 5 mg/mL Sample

1.92 mL Crystallisation screen

14.4 µL Sample $P2_12_12_1$ crystal form seeds (see step 3), dilution 1:250

6 **Crystallisation screen composition:**

[M] 0.1 Molarity (M) MES pH 6.7

11% w/v PEG 4000

5% DMSO

Stock solutions used:

[M] 1 Molarity (M) MES adjusted to pH 6.7 with HCl

50% w/v PEG 4000
99.9% DMSO

Note

The crystallisation screen can be stored in a duran bottle or aliquoted into 96 deep well block for easy dispensing into SwissCI 3 lens plates.

For long term storage keep the crystallisation screen in the fridge at 4°C.

7 Dispense 20 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette. 10m
Dispense 150 nL Sample to each lens using the SPT mosquito.
Dispense 150 nL Crystallisation screen to each lens using the SPT mosquito.
Dispense 50 nL Seeds $P2_12_12_1$ stock to each lens using the SPT mosquito.

Drop ratio: 3:3:1 ratio (150 nL Sample : 150 nL reservoir solution: 50 nl seeds)
Final drop volume: 350 nl

8 Incubate at 20 °C for 24:00:00 h in Formulatrix Rock Imager. 1d

Imaging Schedule: The first images are taken after 12 h and the imaging schedule follows a Fibonacci sequence of days for further collections.

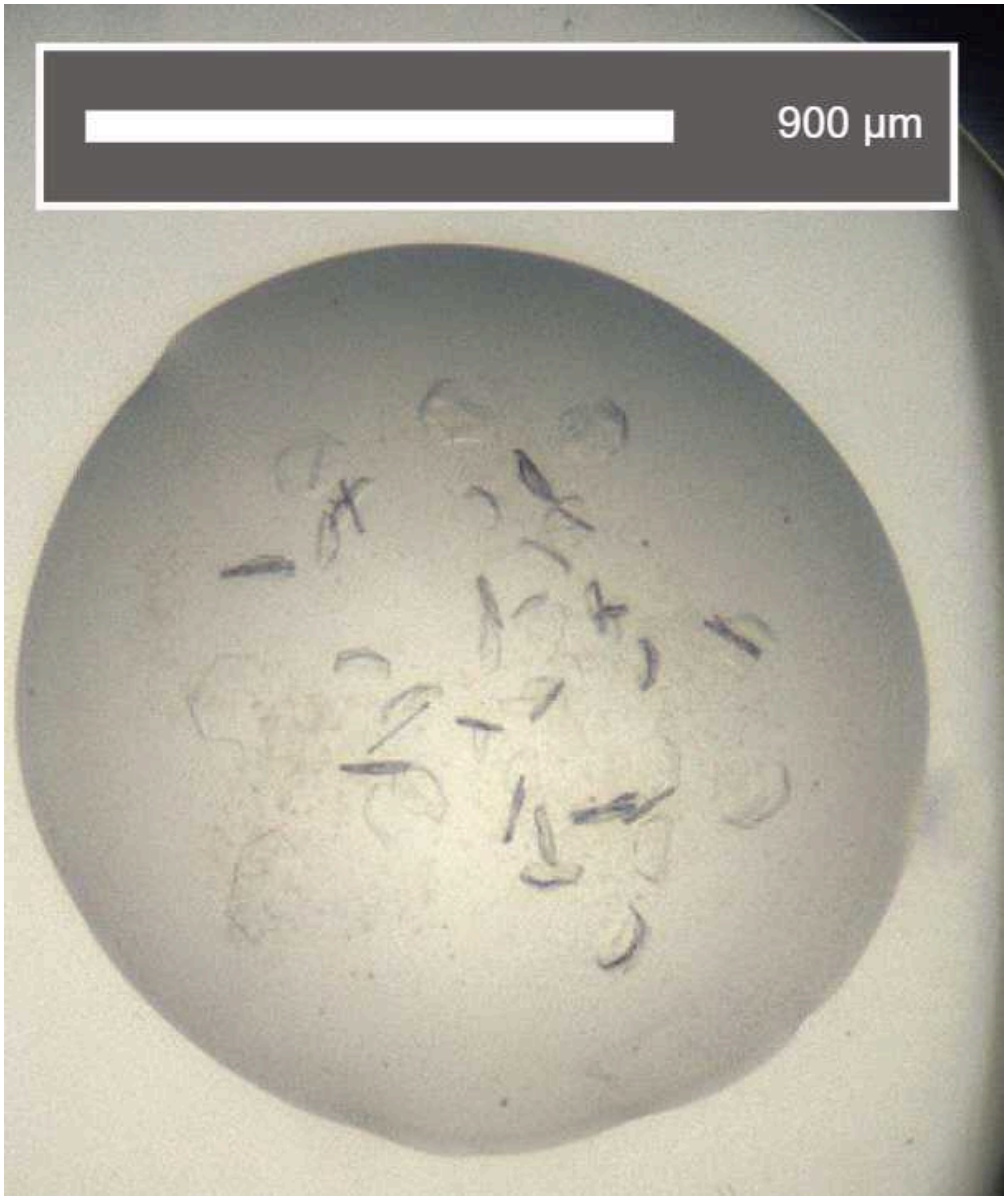
9 Crystal form after ~24 h.

Expected result

The crystals reach their maximum size after 48 h.

Crystals typically form either as stacked playes or in small clusters containng 4-6 crystals.

Morphology: plates.
Size: ~100 µm in length and ~20 µm in width, depth of the crystals is ~10 µm
Appearance: glass shard.
Average resolution: 1.8 Å
Space group: $P2_12_12_1$
Unit cell: 67 Å, 99 Å, 102 Å
90.00°, 90.00°, 90.00°



An example of a drop containing SARS-CoV-2 Mpro protease crystals.

Data collection at Synchrotron

10 Diamond Light Source
Unattended Data Collection (UDC)
Data Collection Temperature: 100K
Detector: DECTRIS EIGER2 X 9M
Beamline: I04-1
Wavelength: 0.9212 Å
Resolution (Å): 1.78
Beam Size (µm): 60 X 50
Number of images: 3600
Oscillation: 0.10°
Exposure (s): 0.0020
Transmission (%): 100
Flux (ph/s): 9.50e+11

Soaking Tolerance

11 Crystals can tolerate up to 20% DMSO, 1-2h

Protocol

NAME

XChem solvent test

CREATED BY

Peter Marples

Preview

Compound Soaking

12 Crystals can tolerate up to 20% DMSO, 1-2h

Protocol

NAME

XChem crystallographic fragment screening

CREATED BY

Peter Marples

Preview

Protocol references

For SARS-CoV-2 Main protease in orthorhombic space group
https://www.rcsb.org/structure/7MBG
https://www.sciencedirect.com/science/article/pii/S0022283621003429#s0045

Group deposition SARS-CoV-2 main protease in complex with inhibitors from the COVID Moonshot
https://www.rcsb.org/structure/7GMB
https://www.science.org/doi/10.1126/science.abo7201