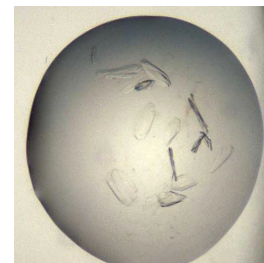


Apr 26, 2024

🌐 Crystallization of SARS-CoV-2 Mpro

DOI

dx.doi.org/10.17504/protocols.io.kqdg326y7v25/v1



blake.h.balcomb^{1,2}, Peter Marples^{1,2}, Lizbé Koekemoer³, Daren Fearon^{1,2}, Charlie Tomlinson^{1,2}

¹Diamond Light Source; ²Research Complex at Harwell; ³Centre of Medicines Discovery, University of Oxford

blake.h.balcomb: The principle crystallographer on the SARS Mpro project.;

ASAP Discovery



Lizbé Koekemoer

University of Oxford

OPEN  ACCESS



DOI: dx.doi.org/10.17504/protocols.io.kqdg326y7v25/v1

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

Protocol Citation: blake.h.balcomb, Peter Marples, Lizbé Koekemoer, Daren Fearon, Charlie Tomlinson 2024. Crystallization of SARS-CoV-2 Mpro. [protocols.io https://dx.doi.org/10.17504/protocols.io.kqdg326y7v25/v1](https://dx.doi.org/10.17504/protocols.io.kqdg326y7v25/v1)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: April 26, 2024

Last Modified: April 26, 2024

Protocol Integer ID: 98844

Keywords: crystallisation, XChem, ASAP, AViDD, CMD, Diamond Light Source, i04-1, SARS CoV, SARS Cov-2 Mpro, Mpro

Funders Acknowledgement:

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.


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  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,  7.5 , [M] 0.5 Molarity (M)
NaCl, 5% glycerol, [M] 0.5 millimolar (mM) TCEP

Safety warnings

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



Equipment needed

- 1 **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

Crystallization experiment

1d

- 2 **Prepare seed stock:**

17m 40s

Protocol



NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

PREVIEW

1: 250 dilution  Sample seeds

- 3 **Protein and buffer requirements:**



43.2 μ L

[M] 5 mg/mL



Sample



1.92 mL Crystallization screen



14.4 µL Sample seeds, dilution 1:250

4 Crystallisation screen composition:

[M] 0.1 Molarity (M) MES 6.7

11% PEG 4000

5% DMSO

Stock solutions used:

[M] 1 Molarity (M) MES adjusted to 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.

5 Dispense 20 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.

10m

Dispense 150 nL [M] 5 mg/mL Sample to each lens using the SPT mosquito.

Dispense 150 nL Crystallisation screen to each lens using the SPT mosquito.

Dispense 50 nL Seeds 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

6 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.

7 Crystal form after ~24 h.

Expected result

The crystals reach their maximum size after 48 h.

Crystals typically form either as stacked plates or in small clusters containing 4-6 crystals.

Morphology: plates.

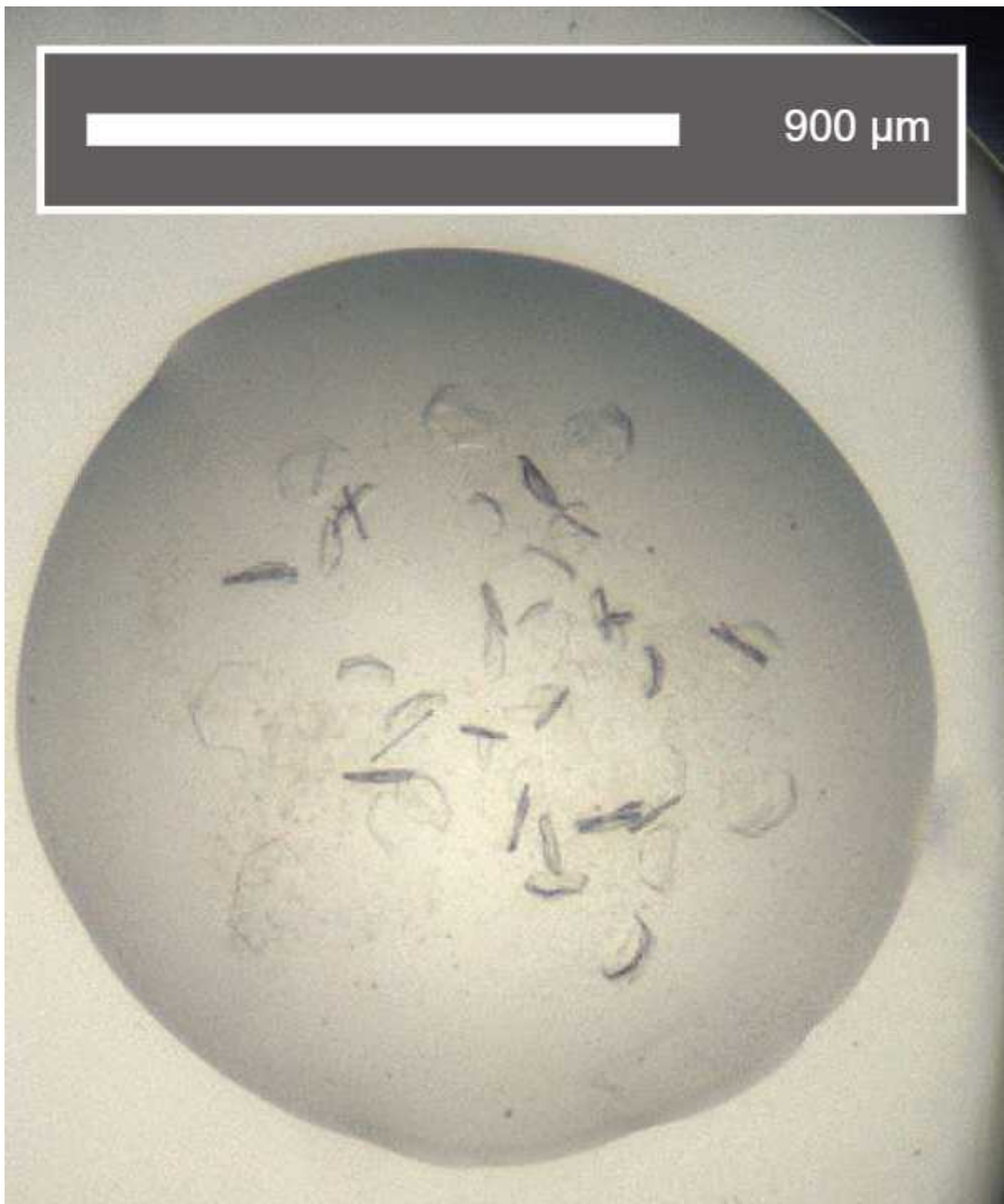
Size: ~100 μm in length and ~20 μm in width, depth of the crystals is ~10 μm

Appearance: glass shard.

Average resolution: 1.8 \AA

Space group: $P2_12_12_1$

Unit cell: 67 \AA , 99 \AA , 102 \AA
90.00°, 90.00°, 90.00°



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

Data collection at Synchrotron

- 8 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

Protocol references

N/A