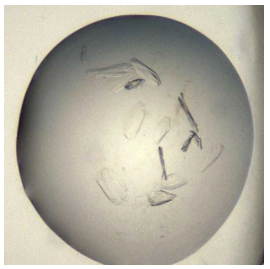
Oct 02, 2024

Crystallisation of SARS-CoV-2 Mpro



Forked from Crystallization of SARS-CoV-2 Mpro

DOI
dx.doi.org/10.17504/protocols.io.261ge5emyg47/v1

blake.h.balcomb[1,2], Peter Marples[1,2], Lizbé Koekemoer[3], Daren Fearon[1,2], Charlie Tomlinson[1,2]

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

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

ASAP Discovery

Peter Marples
Diamond Light Source

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.261ge5emyg47/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. Crystallisation of SARS-CoV-2 Mpro. **protocols.io https://dx.doi.org/10.17504/protocols.io.261ge5emyg47/v1**

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

Created: June 26, 2024

Last Modified: October 02, 2024

Protocol Integer ID: 102454

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 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

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.**

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

Protocol

NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

PREVIEW

1: 250 dilution Sample seeds

4 **Protein and buffer requirements:**

43.2 µL [M] 5 mg/mL Sample

1.92 mL Crystallisation screen

14.4 µL Sample seeds, dilution 1:250

5 **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.

6 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

7 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.

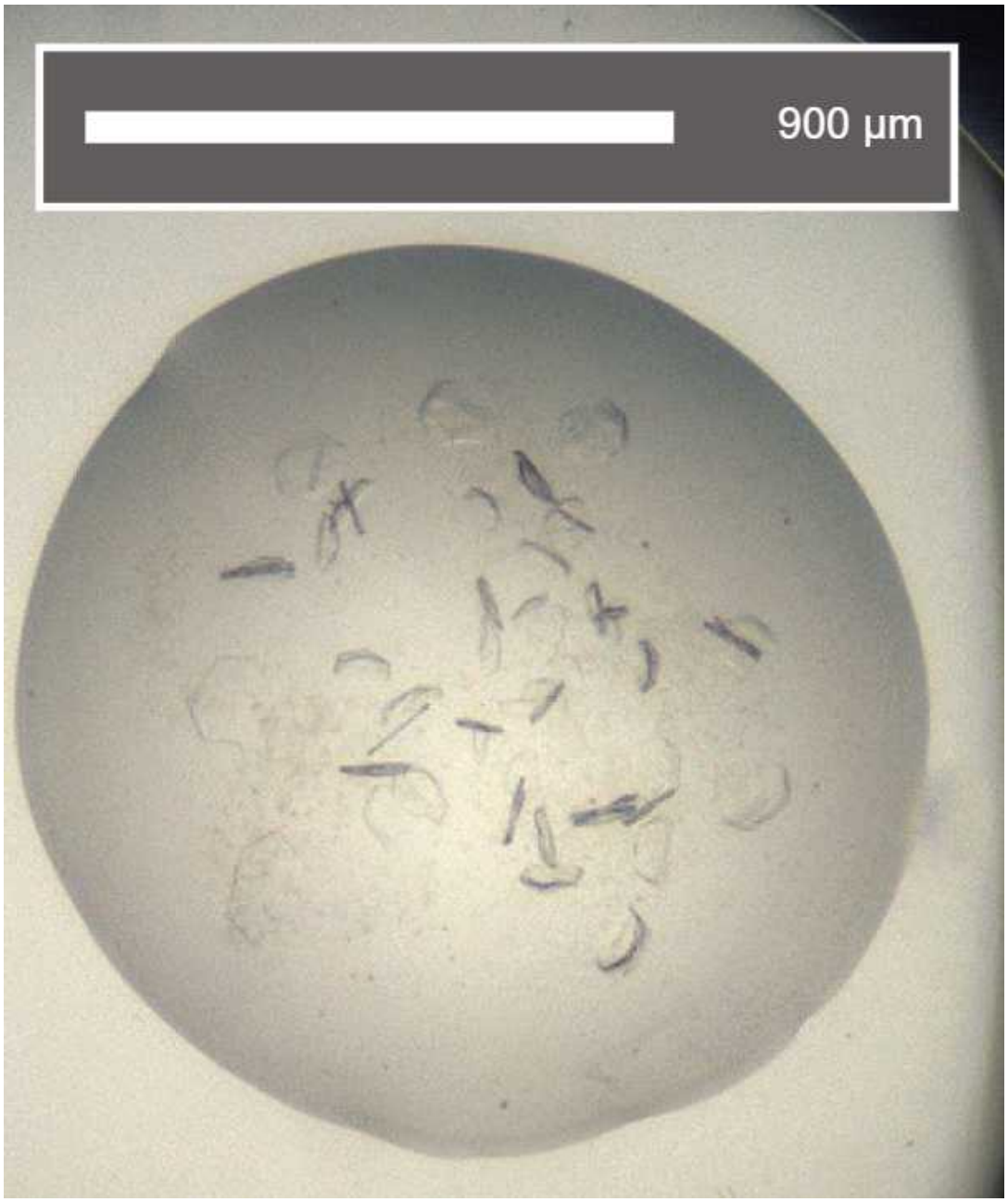
8 Crystal form after ~24 h.

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

9 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