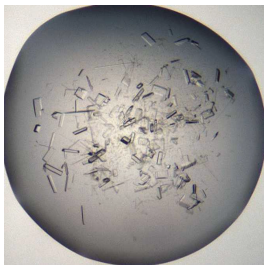
Aug 19, 2024

Crystallisation protocol for SARS-CoV-2 nsp3 macrodomain in P1 21 1



Forked from Crystallization of SARS-CoV-2 Mpro

DOI
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Jasmin Aschenbrenner: The principle crystallographer on the SARS-CoV-2 nsp3 macrodomain project;

ASAP Discovery

Peter Marples
Diamond Light Source

OPEN ACCESS

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External link: https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-SARS-COV-2-NSP3-MAC1

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

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Disclaimer

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

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Abstract

The COVID-19 pandemic has demonstrated the need for novel therapeutic interventions and improved pandemic preparedness strategies against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This protocol details an optimized crystallization method for the SARS-CoV-2 nsp3 macrodomain, a potential drug target. Using sitting drop vapor diffusion with seeding, we describe specific buffer conditions and procedures to consistently produce high-quality crystals suitable for XChem fragment screening. The method yields crystals that diffract to an average resolution of 1.5 Å, enabling high-resolution structural studies and can also be used for compound development through co-crystallization experiments.
All structures solved during the development of tool compounds for the SARS-CoV-2 nsp3 macrodomain are deposited on the PDB (Group deposition: G_1002283).

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 6.5 with HCl, Molecular Dimensions, Catalog # MD2-013-PH 6.5
50% w/v PEG 3000, Molecular Dimensions, Catalog # MD2-100-8

Purified SARS-CoV-2 nsp3 macrodomain protein ([M] 21.6 mg/mL) in [M] 20 millimolar (mM) Tris, pH 7.5 ,
[M] 150 millimolar (mM) NaCl, 5% glycerol, [M] 1 millimolar (mM) TCEP

Safety warnings

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

SARS-CoV-2 nsp3 macrodomain expression and purification

5d

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

Protocol

NAME

SARS-CoV-2 nsp3 macrodomain His-SUMO tagged expression and purification protocol for crystallization (c004)

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

Crystallization experiment

1d

3 **Prepare seed stock:**

17m 40s

Protocol

NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

PREVIEW

1: 2 dilution Sample seeds

4 **Protein and buffer requirements:**

115.2 µL [M] 21.6 mg/mL Sample

2.88 mL Crystallization screen

28.8 µL Sample seeds, dilution 1:2

5 **Crystallisation screen composition:**

[M] 0.1 Molarity (M) MES pH 6.5

30% w/v PEG 3000

Stock solutions used:

[M] 1 Molarity (M) MES adjusted to pH 6.5 with HCl

50% w/v PEG 3000

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 30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette. 10m

Dispense 400 nL [M] 21.6 mg/mL Sample to each lens using the SPT mosquito.

Dispense 300 nL Crystallisation screen to each lens using the SPT mosquito.

Dispense 100 nL Seeds to each lens using the SPT mosquito.

Drop ratio: 4:3:1 ratio (400 nl Sample : 300 nl reservoir solution: 100 nl seeds)

Final drop volume: 800 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.

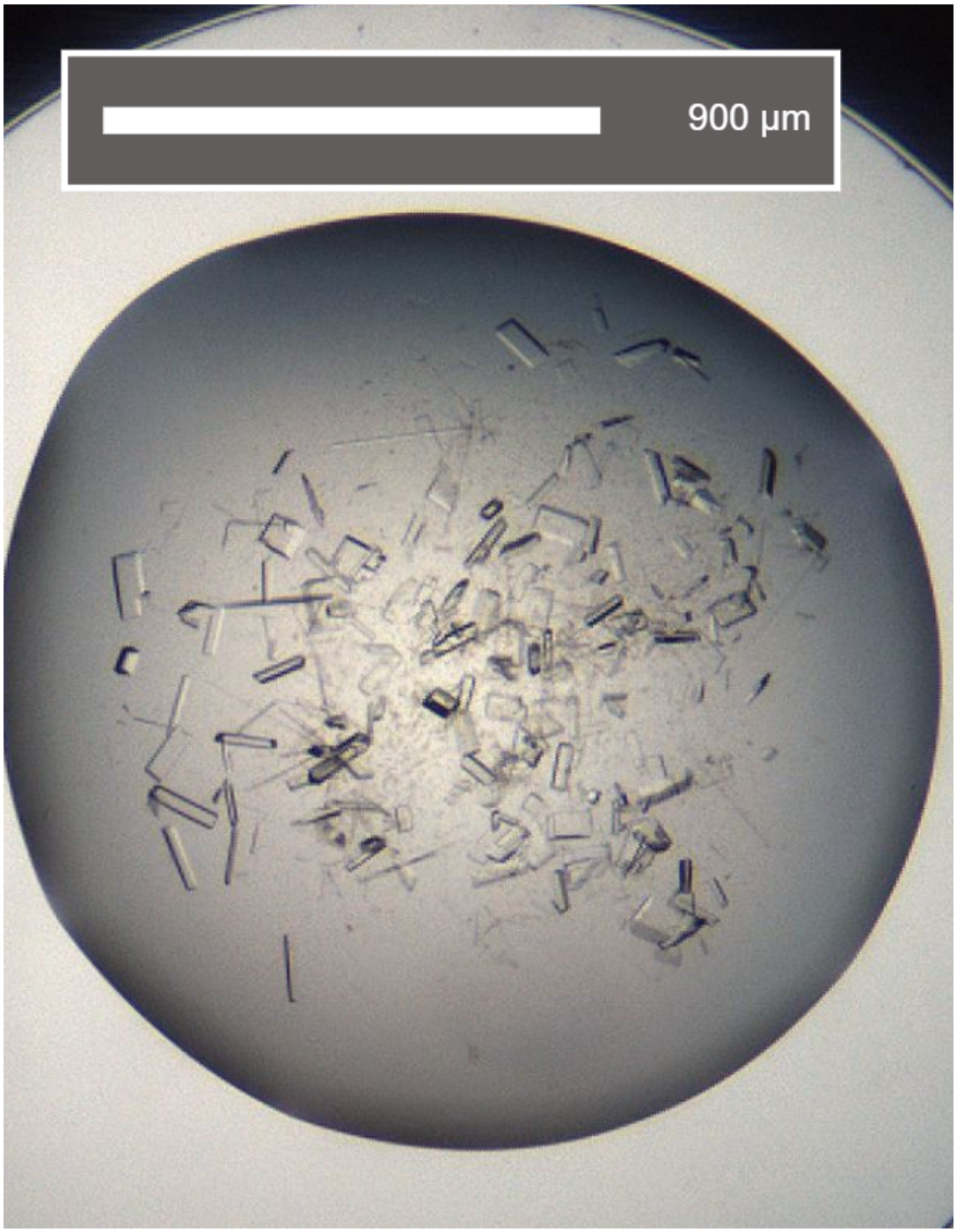
8 Crystal form after ~12 h.

Expected result

The crystals reach their maximum size after 24 h.

Crystals typically form either as plates or as long rods.

Morphology: plates / rods.
Size: ~50 µm in length and ~50 µm in width, depth of the crystals is ~20 µm / ~70 µm in length and ~10 µm in width, depth of the crystals is ~10 µm
Appearance: rectangular or rods.
Average resolution: 1.5 Å
Space group: $P12_11$
Unit cell: 37 Å, 33 Å, 61 Å
90.00°, 96.00°, 90.00°



An example of a drop containing SARS-CoV-2 nsp3 macrodomain 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): 1.62
Beam Size (µm): 60 X 50
Number of images: 3600
Oscillation: 0.10°
Exposure (s): 0.0020
Transmission (%): 100
Flux (ph/s): 3.80e+12

Protocol references

N/A