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



Forked from [Crystallization of SARS-CoV-2 Mpro](#)

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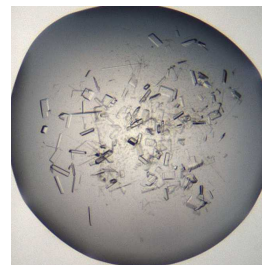
Jasmin Aschenbrenner: The principle crystallographer on the SARS-CoV-2 nsp3 macrodomain project;

ASAP Discovery



Peter Marples

Diamond Light Source



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DOI: <https://dx.doi.org/10.17504/protocols.io.261ge5ej7g47/v1>

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  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,  7.5 ,
[M] 150 millimolar (mM) NaCl, 5% glycerol, [M] 1 millimolar (mM) TCEP

Troubleshooting

Safety warnings

 Follow all handling warning for the chemicals used in the crystallisation 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 μ L 21.6 mg/mL Sample

2.88 mL Crystallization screen

 28.8 μ L Sample seeds, dilution 1:2

5 Crystallisation screen composition:

0.1 Molarity (M) MES 6.5

30% w/v PEG 3000

Stock solutions used:

1 Molarity (M) MES adjusted to 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 μ L Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 μ l multi-channel pipette.

10m

Dispense 400 nL 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.

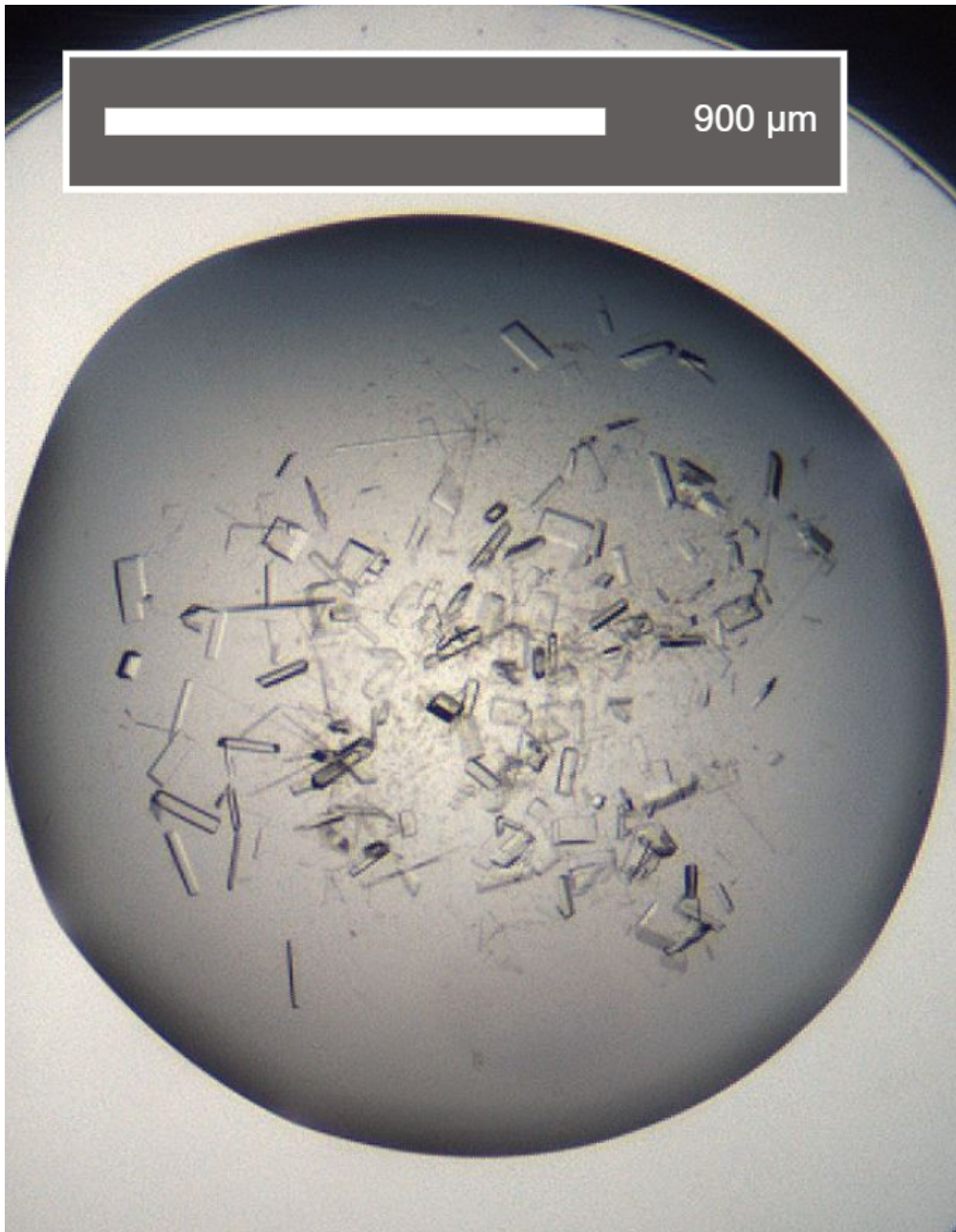
Size: ~50 μm in length and ~50 μm in width, depth of the crystals is ~20 μm / ~70 μm in length and ~10 μm in width, depth of the crystals is ~10 μm

Appearance: rectangular or rods.

Average resolution: 1.5 $\text{\AA}$

Space group: P12₁1

Unit cell: 37 $\text{\AA}$, 33 $\text{\AA}$, 61 $\text{\AA}$
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