



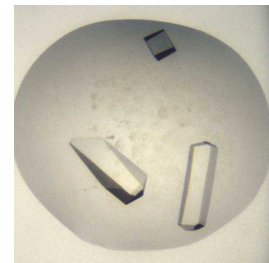
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Version 2

Crystallisation protocol for SARS-CoV-2 nsp3 macrodomain in P43 V.2

DOI

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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, 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 1.2 Å, enabling high-resolution structural studies.

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). In this version we added the Addgene id and changed the results section.

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) CHES, pH 9.5, Molecular Dimensions, Catalog # MD2-008-PH 9.5

50% w/v PEG 3000, Molecular Dimensions, Catalog # MD2-100-8

Purified SARS-CoV-2 nsp3 macrodomain protein ([M] 47 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

 Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolate:Wuhan-Hu-1 nsp3 macrodomain **addgene** Catalog #228643

Safety warnings

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



SARS-CoV-2 nsp3 macrodomain expression and purification

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



Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) isolate:Wuhan-Hu-1 nsp3 macrodomain **addgene Catalog #228643**

Protocol

NAME

SARS-CoV-2 nsp3 macrodomain expression and purification protocol for crystallization

CREATED BY

korvus.wang

Preview

Equipment needed

- 2 **Formulatrix Rock Imager** (or incubator of choice)
SPT mosquito
P100 multi-channel pipette
SwissCI 3 lens plate

Crystallization experiment

1d

- 3 **Protein and buffer requirements:**



45 μ L

[M] 47 mg/mL



Sample



2.0 mL Crystallization screen

- 4 **Crystallisation screen composition:**



[M] 100 millimolar (mM)

CHES,



9.5

30% w/v PEG 3000

**Stock solutions used:**

[M] 1 Molarity (M) CHES adjusted to pH 9.5 with NaOH

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.

- 5 Dispense $\text{20 } \mu\text{L}$ Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 μL multi-channel pipette.

Dispense $\text{150 } \mu\text{L}$ [M] 47 mg/mL Sample to each lens using the SPT mosquito.

Dispense $\text{150 } \mu\text{L}$ Crystallisation screen to each lens using the SPT mosquito.

Drop ratio: 1:1 ratio (150 nL Sample : 150 nL reservoir solution)

Final drop volume: 300 nL

- 6 Incubate at $\text{20 } ^\circ\text{C}$ for 48:00:00 h in Formulatrix Rock Imager.

2d

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 as single crystals.

Morphology: typically large cubes or rectangles.

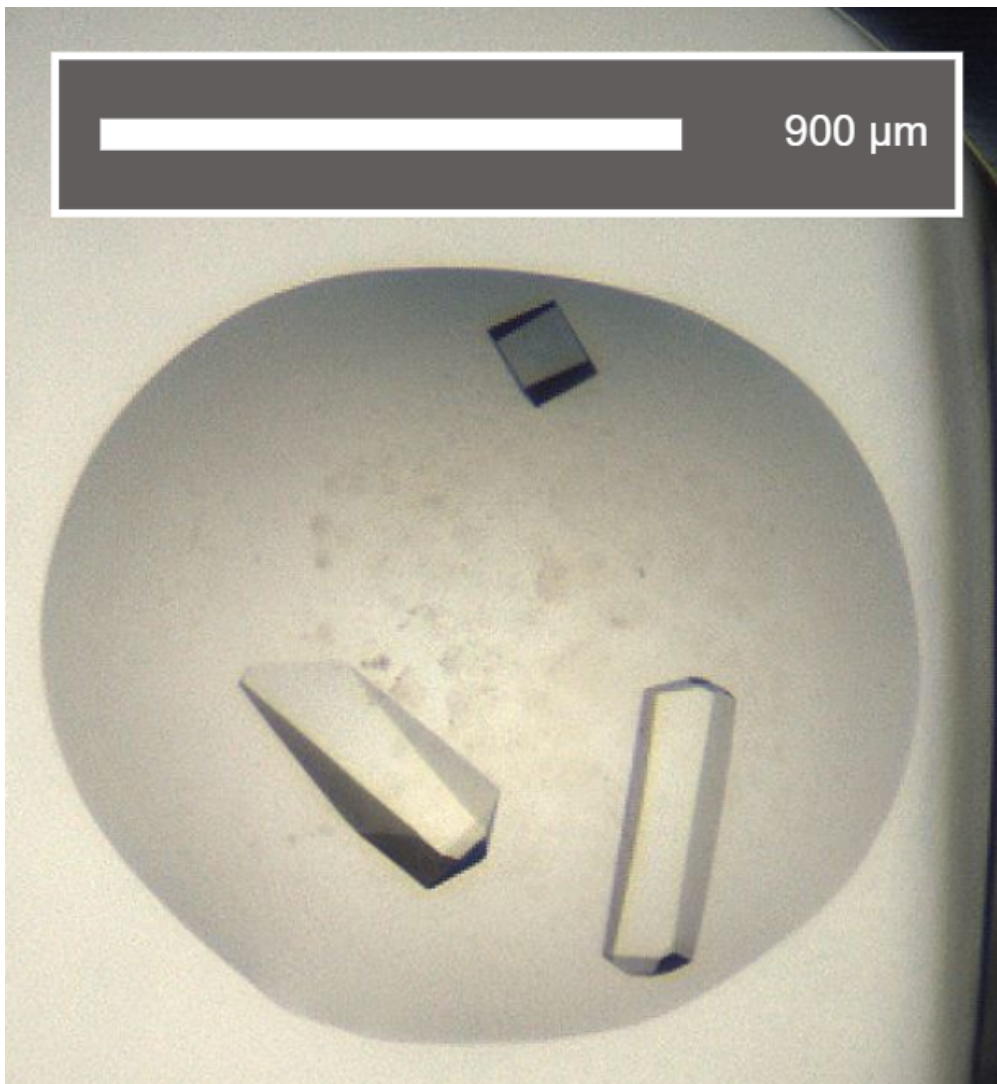
Size: ~300 μm in length and ~150 μm in width, depth of the crystals is ~100 μm

Appearance: large crystal chunks.

Average resolution: 1.2 $\text{\AA}$

Space group: $P4_3$

Unit cell: 89 $\text{\AA}$, 89 $\text{\AA}$, 39 $\text{\AA}$
90.00°, 90.00°, 90.00°



An example of a drop containing SARS-CoV-2 nsp3 macrodomain 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): 1.62
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

Protocol based on

Marion Schuller *et al.*, Fragment binding to the Nsp3 macrodomain of SARS-CoV-2 identified through crystallographic screening and computational docking. *Sci. Adv.* **7**, eabf8711 (2021).

DOI: [10.1126/sciadv.abf8711](https://doi.org/10.1126/sciadv.abf8711)