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## 🌐 Co-crystallisation protocol for SARS-CoV-2 nsp3 macrodomain in P1 21 1 with ligands

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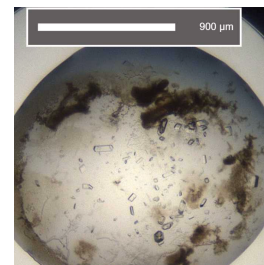
Jasmin Aschenbrenner: The principle crystallographer on the SARS-CoV-2 nsp3 macrodomain project;

ASAP Discovery



Lizbé Koekemoer

University of Oxford



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DOI: [dx.doi.org/10.17504/protocols.io.n2bvjnb9bgk5/v1](https://dx.doi.org/10.17504/protocols.io.n2bvjnb9bgk5/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**

**Created:** August 15, 2024

**Last Modified:** August 19, 2024

**Protocol Integer ID:** 105615

**Keywords:** crystallisation, XChem, ASAP, AViDD, CMD, Diamond Light Source, i04-1, SARS-CoV-2, nsp3, macrodomain, co-crystallisation

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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 produce high-quality crystals of ligand-bound nsp3-mac.

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  $\text{pH}$  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,  $\text{pH}$  7.5 ,  
[M] 150 millimolar (mM) NaCl, 5% glycerol, [M] 1 millimolar (mM) TCEP

Compounds ( [M] 100 millimolar (mM) ) in DMSO

## 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.**

### 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 **Echo liquid handler**  
**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/><sup>LINK</sup>

P100 8 multi-channel pipette

**SwissCI 3 lens plate**

## Crystallization experiment

1d



### 3 Prepare seed stock:

#### Protocol



NAME





#### Diamond XChem Seeding Protocol

CREATED BY



Peter Marples

PREVIEW1: 2 dilution  Sample seeds

### 4 Protein, compounds and buffer requirements:



 115.2  $\mu$ L  21.6 mg/mL  Sample 2.88 mL Crystallization screen 28.8  $\mu$ L  Sample seeds, dilution 1:2 12  $\mu$ L  100 millimolar (mM)  Sample ligand in DMSO

### 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  $\mu$ L Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100  $\mu$ l multi-channel pipette.

Dispense  40 nL Compound (~ 5% of final drop volume) to each lens using the Echo liquid handler.



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:** 400 nL Sample : 300 nL reservoir solution: 100 nL seeds: 40 nL compounds

**Final drop volume:** 840 nL

- 7 Incubate at 20 °C for 24:00:00 h to maximum of 48:00:00 h in Formulatrix Rock Imager.

3d

**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.

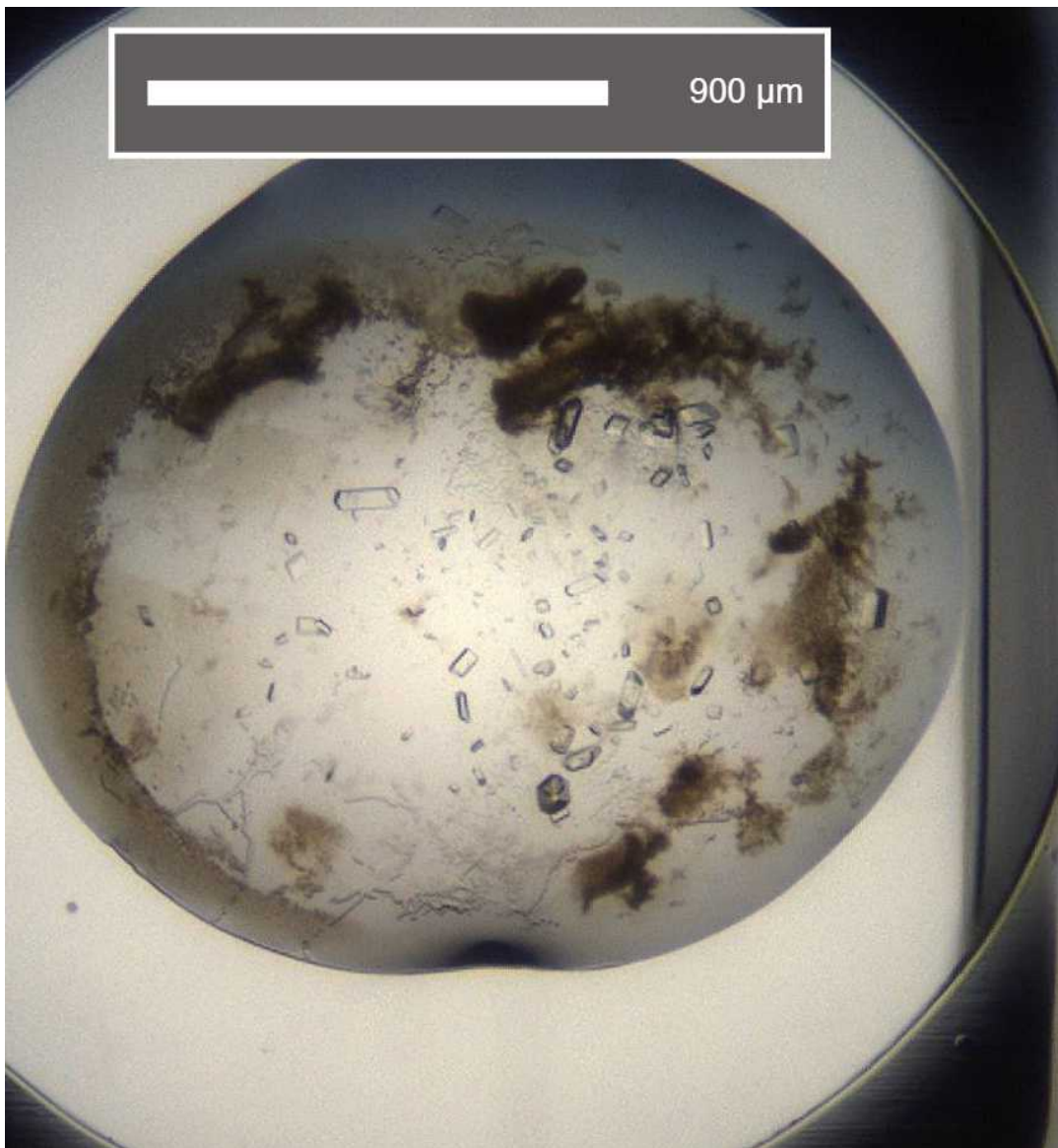
**Size:** ~50  $\mu\text{m}$  in length and ~50  $\mu\text{m}$  in width, depth of the crystals is ~20  $\mu\text{m}$  / ~70  $\mu\text{m}$  in length and ~10  $\mu\text{m}$  in width, depth of the crystals is ~10  $\mu\text{m}$

**Appearance:** rectangular or rods. Compound will often cause some precipitation in drop

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

**Space group:**  $P12_11$

**Unit cell:** 37  $\text{\AA}$ , 33  $\text{\AA}$ , 61  $\text{\AA}$   
90.00°, 96.00°, 90.00°



An example of a co-crystallisation drop containing SARS-CoV-2 nsp3 macrodomain crystals with compound.

## 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