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Crystallisation of SARS-CoV-2 N Protein V.1

Forked from a private protocol

DOI
https://dx.doi.org/10.17504/protocols.io.8epv5r8ydg1b/v1

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ASAP Discovery

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

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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 crystallization protocol and buffer conditions used to obtain reproducible SARS C0V-2 Nucelocapsid 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) HEPES adjusted to pH 7.8 with NaOH, Molecular Dimensions, Catalog # MD2-011-PH 7.8
70 % isopropanol, Sigma Aldrich, Catalog # **563935**
50% w/v PEG 4000, Molecular Dimensions, Catalog # MD2-250-11

Purified SARS CoV-2 Nucleocapsid protein ([M] 20 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

Construct used protein residues 250-364

Safety warnings

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

Equipment needed

1 **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

2 **Prepare seed stock:**

Protocol

NAME
Diamond XChem Seeding Protocol

CREATED BY
Peter Marples

Preview

1: 100 dilution Sample seeds

3 **Protein and buffer requirements:**
57.6 µL [M] 20 mg/mL Sample

2.88 mL Crystallization screen
5.76 µL Sample seeds, dilution 1:100

4 **Crystallisation screen composition:**
[M] 0.1 Molarity (M) HEPES pH 7.8
10 % isopropanol
23% w/v PEG 4000

Stock solutions used:
[M] 1 Molarity (M) HEPES adjusted to pH 7.8 with NaOH
70 % isopropanol
50% w/v PEG 4000

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 30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.
Dispense 200 nL [M] 20 mg/mL Sample to each lens using the SPT mosquito.
Dispense 180 nL Crystallisation screen to each lens using the SPT mosquito.
Dispense 20 nL Seeds to each lens using the SPT mosquito.

Drop ratio: 5:9:1 ratio (100 nL Sample : 180 nL reservoir solution: 20 nL seeds)
Final drop volume: 300 nl

6 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.

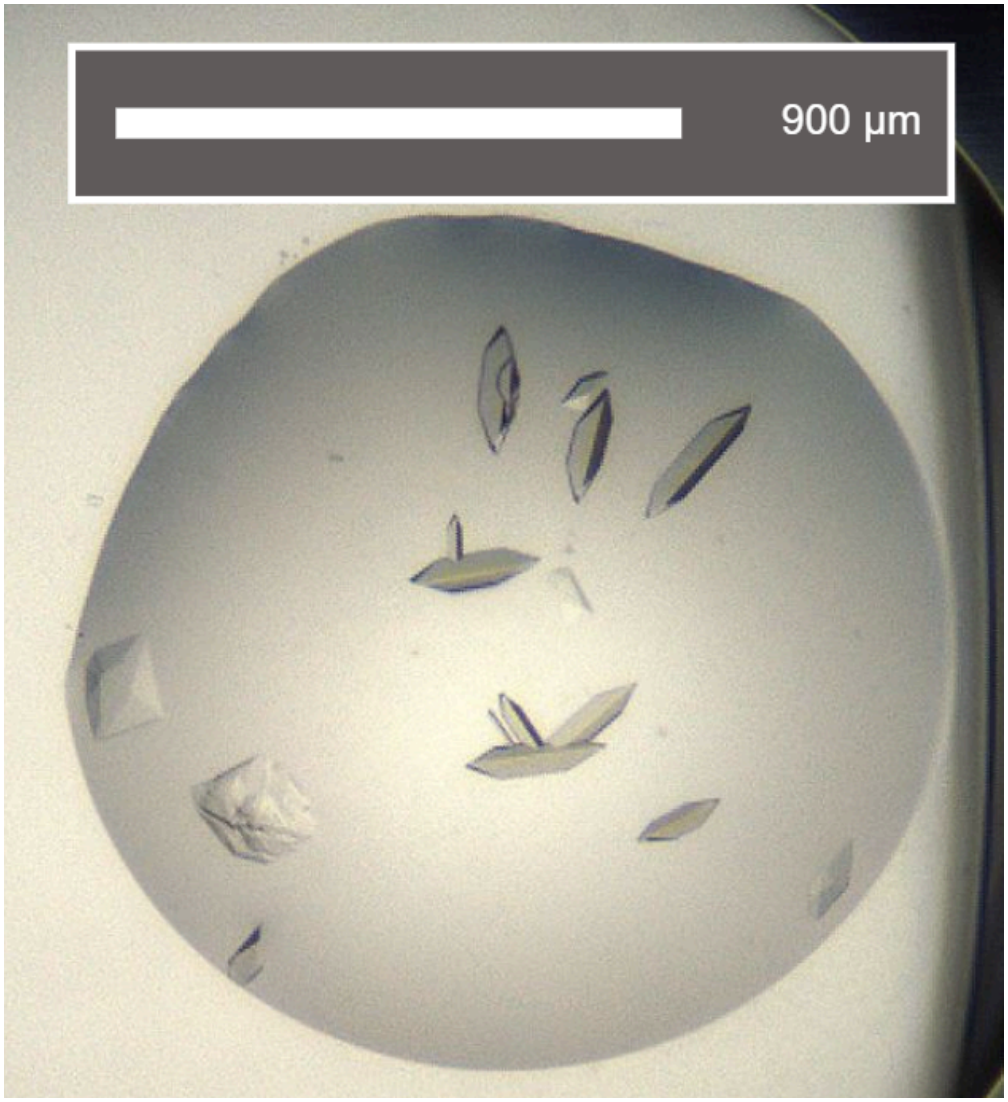
7 Crystal form after ~12 h.

Expected result

The crystals reach their maximum size after 24 h.

Crystals typically form as single crystals as six sided shards

Morphology: six sided shard
Size: ~100 µm in length and ~30 µm in width, depth of the crystals is ~30 µm
Appearance: glass shard.
Average resolution: 2.0 Å
Space group: $I4_1$
Unit cell: 89, 89, 40
90.00°, 90.00°, 90.00°



An example of a drop containing SARS N-protein 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): 1.68
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