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Version 1

READDI protocol: Crystallisation of CHIKV nsP3 macrodomain V.1

Forked from a private protocol

DOI

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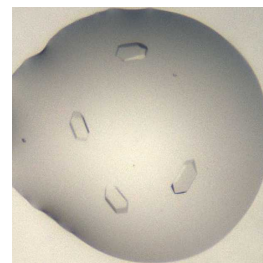
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ASAP Discovery



Peter Marples

Diamond Light Source



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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 Chikungunya Virus NS3 macrodomain crystals suitable for **XChem** fragment screening.

Guidelines

N/A



Materials

SwissCI 3 lens crystallization plates <https://swissci.com/product/3-lens-crystallisation-plate/> **Codes:**

Midi: UVXPO-3LENS 3W96T-PS 3W96T-UVP

Molecular Dimensions 'The BCS Screen Single Reagent' 2-44:

0.1 M Tris (pH 7.8), 0.1 M Potassium thiocyanate, 0.1 M Sodium bromide, 25 % v/v PEG Smear Broad, Catalog # MDSR-104-2-44

Purified CHIKV Mac protein (11 mg/mL) in 25 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 5 % Glycerol.

Troubleshooting

Safety warnings

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

Ethics statement

N/A



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:**

17m 40s

Protocol



NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

Preview

1: 100 dilution  Sample seeds

- 3 **Protein and buffer requirements:**



21.6 μ L



[M] 11 mg/mL



Sample



🧪 2.88 mL Crystallisation screen

🧪 10.08 µL Sample Seeds, dilution 1:100

4 Crystallisation screen composition:

[M] 0.1 Molarity (M) Tris-NaOH 7.8

[M] 0.1 Molarity (M) Potassium thiocyanate

[M] 0.1 Molarity (M) Sodium bromide

25 % v/v PEG Smear Broad

Stock solutions used:

[M] 1 Molarity (M) Tris adjusted to 7.8 with NaOH

[M] 1 Molarity (M) Potassium thiocyanate

[M] 1 Molarity (M) Sodium bromide

50% v/v PEG Smear Broad

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.

10m

Dispense 75 µL [M] 11 mg/mL Sample to each lens using the SPT mosquito.

Dispense 40 µL Crystallisation screen to each lens using the SPT mosquito.

Dispense 35 µL CHIKV Mac Seeds to each lens using the SPT mosquito.

Drop ratio: 15:8:7 ratio (75 nl Sample : 40 nl Crystallisation solution: 35 nl Seeds)

Final drop volume: 150 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

Expected result

The crystals reach their maximum size after 24-48 h.

Crystals typically form as single crystals at the bottom of the drop or on the drop-air interface.

Morphology: typically thin rectangles with pointed ends.

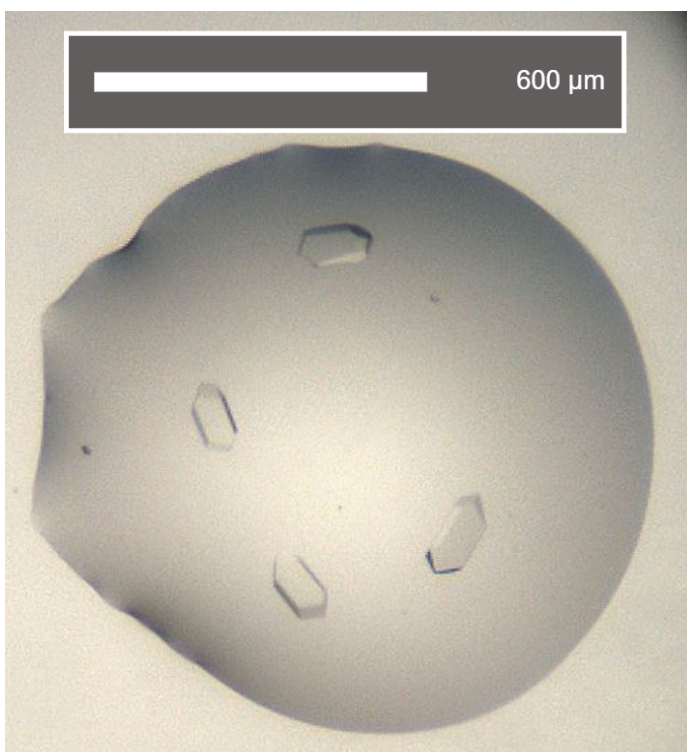
Size: ~100 μm in length and ~50 μm in width, depth of the crystals is ~10 μm

Appearance: glass shard.

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

Space group: $P3_1$

Unit cell: 87 $\text{\AA}$, 87 $\text{\AA}$, 85 $\text{\AA}$
90.00°, 90.00°, 120.00°



An example of a drop containing CHIKV 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): 1.64

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

Identifying novel chemical matter against the Chikungunya virus nsP3 macrodomain through crystallographic fragment screening, <https://doi.org/10.1101/2024.08.23.609196>