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Version 2

🌐 Crystallization of Zika virus NS3 helicase PDB V.2

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External link: <https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-ZIKA-NS3-HELICASE>

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We use this protocol and it's working

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Disclaimer

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Oxford Lab Technologies crystal shifter <https://doi.org/10.1107/S2059798320014114>

Abstract

The crystallization protocol and buffer conditions used to obtain Zika NS3 helicase crystals suitable for **XChem** fragment screening. The Zika virus (ZIKV), discovered in Africa in 1947, swiftly spread across continents, causing significant concern due to its recent association with microcephaly in newborns and Guillain-Barré syndrome in adults. Despite a decrease in prevalence, the potential for a resurgence remains, necessitating urgent therapeutic interventions. Like other flaviviruses, ZIKV presents promising drug targets within its replication machinery, notably the NS3 helicase (NS3Hel) protein, which plays critical roles in viral replication. However, a lack of structural information impedes the development of specific inhibitors targeting NS3Hel. This protocol was used to grow Zika NS3 crystals that were applied high-throughput crystallographic fragment screening on ZIKV NS3 Helicase. In this new version, we added the Addgene information for the construct used for heterologous protein expression and the protocol for protein production and purification

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) NPS Mix (consisting of 0.3 M Sodium phosphate dibasic dihydrate, 0.3 M Ammonium sulphate, and 0.3 M Sodium nitrate from Molecular Dimensions), Catalog # MD2-250-72

[M] 1 Molarity (M) MES, Molecular Dimensions, Catalog # MD2-013-PH

Precipitant Mix 4 (11% MPD, 11% PEG 1,000, and 11% PEG 3,350 from Molecular Dimensions), Catalog # MD2-250-84

Purified Zika NS3 protein ([M] 5 mg/mL) in [M] 20 millimolar (mM) Bis-Tris  7 , [M] 500 millimolar (mM) NaCl, 10% glycerol

Protocol materials

 Zika virus (ZIKV) isolate PRVABC59 NS3 helicase domain **addgene** Catalog #228664

Safety warnings

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



Protein expression and purification

- 1 The protein expression and purification of the plasmid



Zika virus (ZIKV) isolate PRVABC59 NS3 helicase domain **addgene Catalog #228664**

was done following the protocol below.

Equipment needed

- 2 **Formulatrix Rock Imager** (or incubator of choice)
SPT mosquito
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

Preview

1: 100 dilution Sample seeds

- 4 **Protein and buffer requirements:**



43.2 μ L

[M] 5 mg/mL



Sample



4.8 mL Crystallization screen



4.8 mL seeds, dilution 1:100

- 5 **Crystallisation screen composition:**



0.12 Molarity (M)

NPS Mix



[M] 0.1 Molarity (M) MES  6.5

33% Precipitant Mix 4

Stock solutions used:

[M] 1 Molarity (M) NPS Mix (consisting of 0.3 M Sodium phosphate dibasic dihydrate, 0.3 M Ammonium sulphate, and 0.3 M Sodium nitrate from Molecular Dimensions)

[M] 1 Molarity (M) MES (Molecular Dimensions)

Precipitant Mix 4 (11% MPD, 11% PEG 1,000, and 11% PEG 3,350 from Sigma Aldrich)

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  50 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.

Dispense  150 µL [M] 5 mg/mL  Sample to each lens using the SPT mosquito.

Dispense  100 µL Crystallisation screen to each lens using the SPT mosquito.

Dispense  50 µL Seeds to each lens using the SPT mosquito.

Drop ratio: 3:2:1 ratio (150 nL  Sample : 100 nL reservoir solution: 50 nL seeds)

Final drop volume: 300 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

Expected result

The crystals reach their maximum size after 48 h.

Crystals typically form either as single crystals or overlapping thin plates

Morphology: typically thin square plates

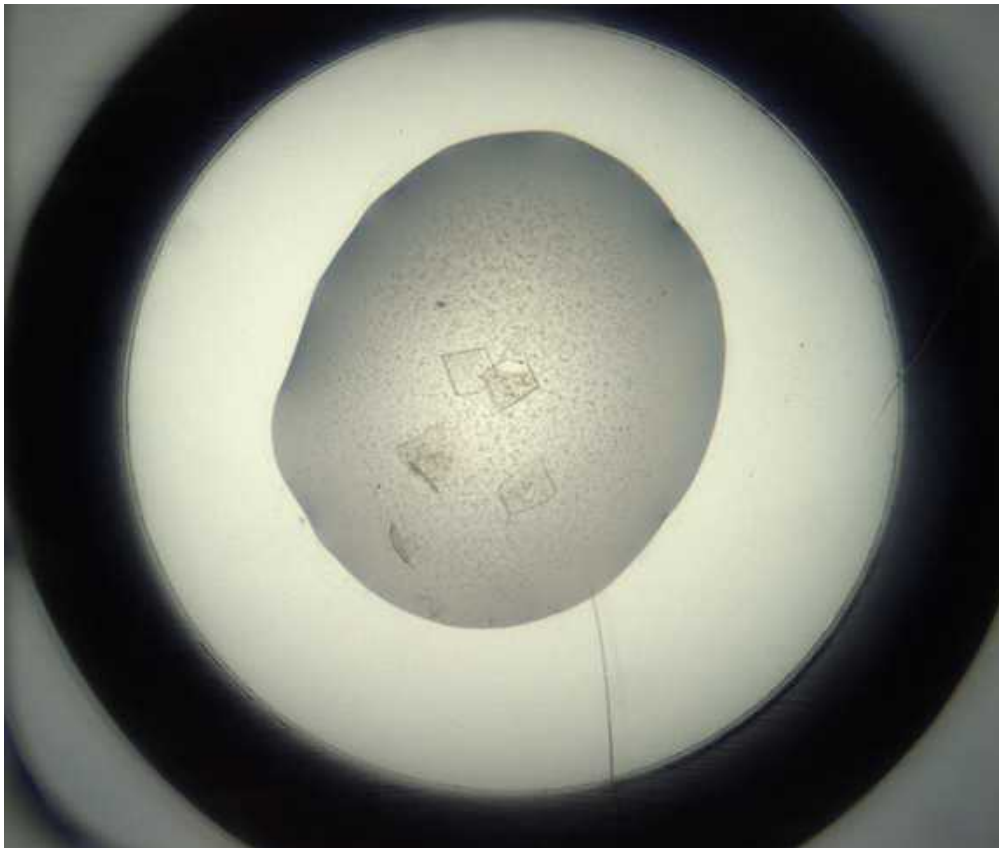
Size: ~100 μm in length and ~100 μm in width, depth of the crystals is ~10 μm

Appearance: glass shard.

Average resolution: 1.8 $\text{\AA}$

Space group: P12₁

Unit cell: 53 $\text{\AA}$, 69 $\text{\AA}$, 57 $\text{\AA}$
90.00°, 92.00°, 90.00°



An example of a drop containing Zika NS3 helicase 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

Solvent tolerance

10 Crystals were tolerant at 20% DMSO for 2hs

Protocol



NAME

XChem solvent test

CREATED BY

Peter Marples

Preview

Fragment Screen

11 The compounds were soaked at 20% DMSO for 2hs



Protocol



NAME

XChem crystallographic fragment screening

CREATED BY

Peter Marples

Preview

Protocol references

N/A