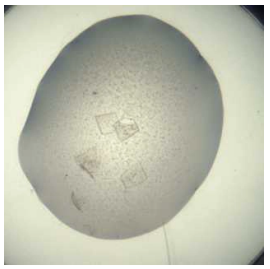
Apr 26, 2024

Crystallization of Zika virus NS3 helicase



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ASAP Discovery

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

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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 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.

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 pH 7 , [M] 500 millimolar (mM) NaCl, 10% glycerol

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

Crystallization 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:**
43.2 µL [M] 5 mg/mL Sample
4.8 mL Crystallization screen

4.8 mL seeds, dilution 1:100

4 **Crystallisation screen composition:**

[M] 0.12 Molarity (M) NPS Mix

[M] 0.1 Molarity (M) MES pH 6.5

33% Prcipitant 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.

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

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

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

Dispense 50 undetermined 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

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.

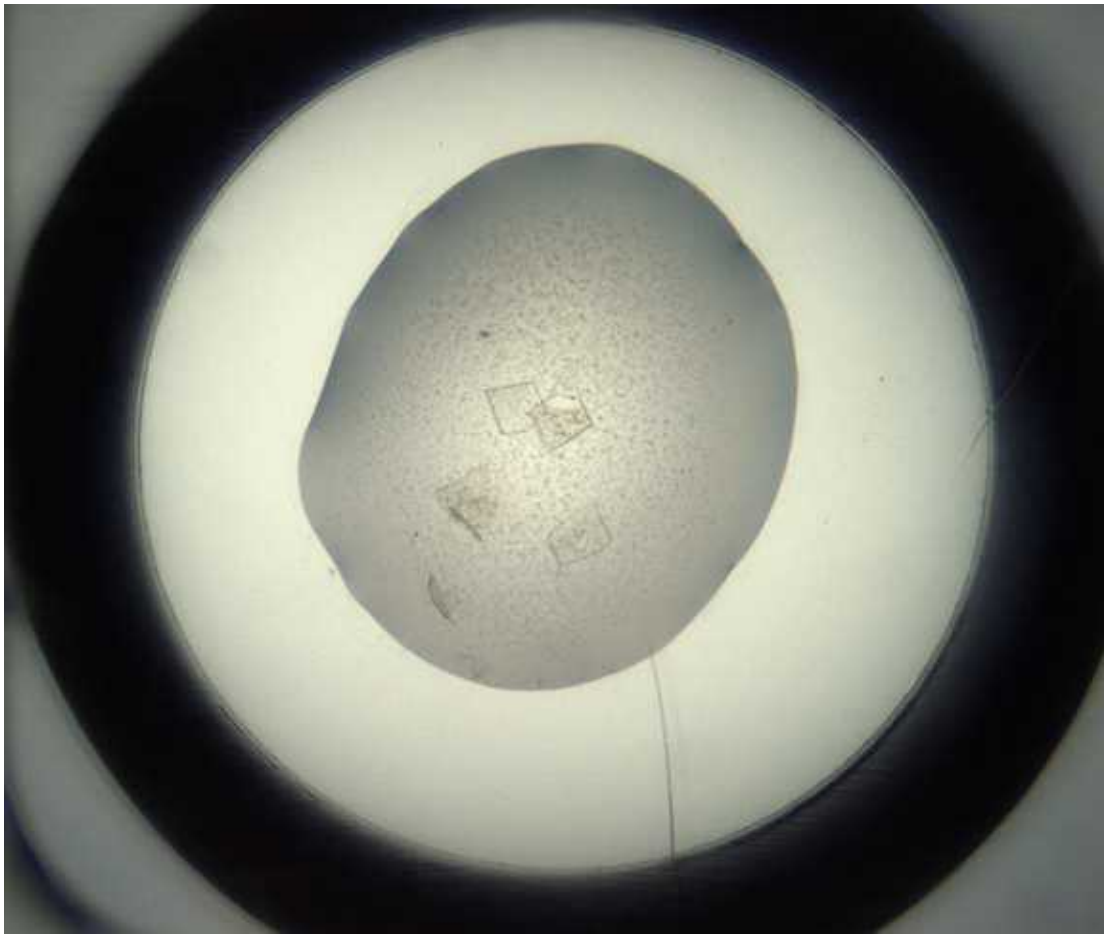
7

Expected result

The crystals reach their maximum size after 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 Å
Space group: $P12_1$
Unit cell: 53 Å, 69 Å, 57 Å
90.00°, 92.00°, 90.00°



An example of a drop containing Zika NS3 helicase 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

N/A