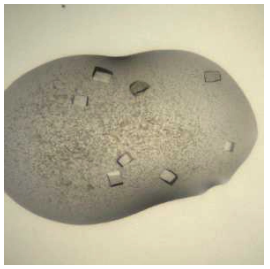
Apr 26, 2024

Crystallization of Zika virus NS2B-NS3 protease



Forked from ASAP protocol: Crystallization of Enterovirus A71 3C protease (PDB code 8CNY)

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ASAP Discovery

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DOI: dx.doi.org/10.17504/protocols.io.eq2lyj51mlx9/v1

External link: https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-ZIKA-NS2B-NS3

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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

Zika virus (ZIKV) infections causes microcephaly in new-borns and Guillain-Barre syndrome in adults raising a global public health concern, yet no vaccines or antiviral drugs are available to treat or prevent ZIKV infections. The viral NS3 protease with its NS2B cofactor is essential for the cleavage of Zika polyprotein precursor into individual structural and non-structural proteins and is therefore an attractive drug target. We optimized a robust crystal system of co-expressed NS3 protease with its NS2B cofactor and used this in a crystallographic fragment screening campaign.

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) Ammonium sulfate, Molecular Dimensions, Catalog # MD2-250-35

[M] 1 Molarity (M) Sodium acetate pH 4.8 , Molecular Dimensions, Catalog # 133225

50% w/v PEG 2000, Molecular Dimensions, Catalog # MD2-250-17

Purified Zika NS2BNS3 protein ([M] 15 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

Protein construct **https://www.addgene.org/204791/**

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 **Protein and buffer requirements:**
43.2 µL | [M] 15 mg/mL | Sample
2.88 mL Crystallization screen

3 **Crystallisation screen composition:**
30% w/v PEG 2000
[M] 0.2 Molarity (M) Ammonium sulfate
[M] 0.1 Molarity (M) sodium acetate pH 4.8

Stock solutions used:
[M] 1 Molarity (M) Ammonium sulfate
[M] 1 Molarity (M) Sodium acetate pH 4.8
50% w/v PEG 2000

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.

4 Dispense 30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.
Dispense 150 nL nl [M] 15 mg/mL Sample to each lens using the SPT mosquito.
Dispense 150 nL Crystallisation screen to each lens using the SPT mosquito.

Drop ratio: 1:1
Final drop volume: 300 nl

5 Incubate at 20 °C for 24:00:00 h in Formulatrix Rock Imager. 1d

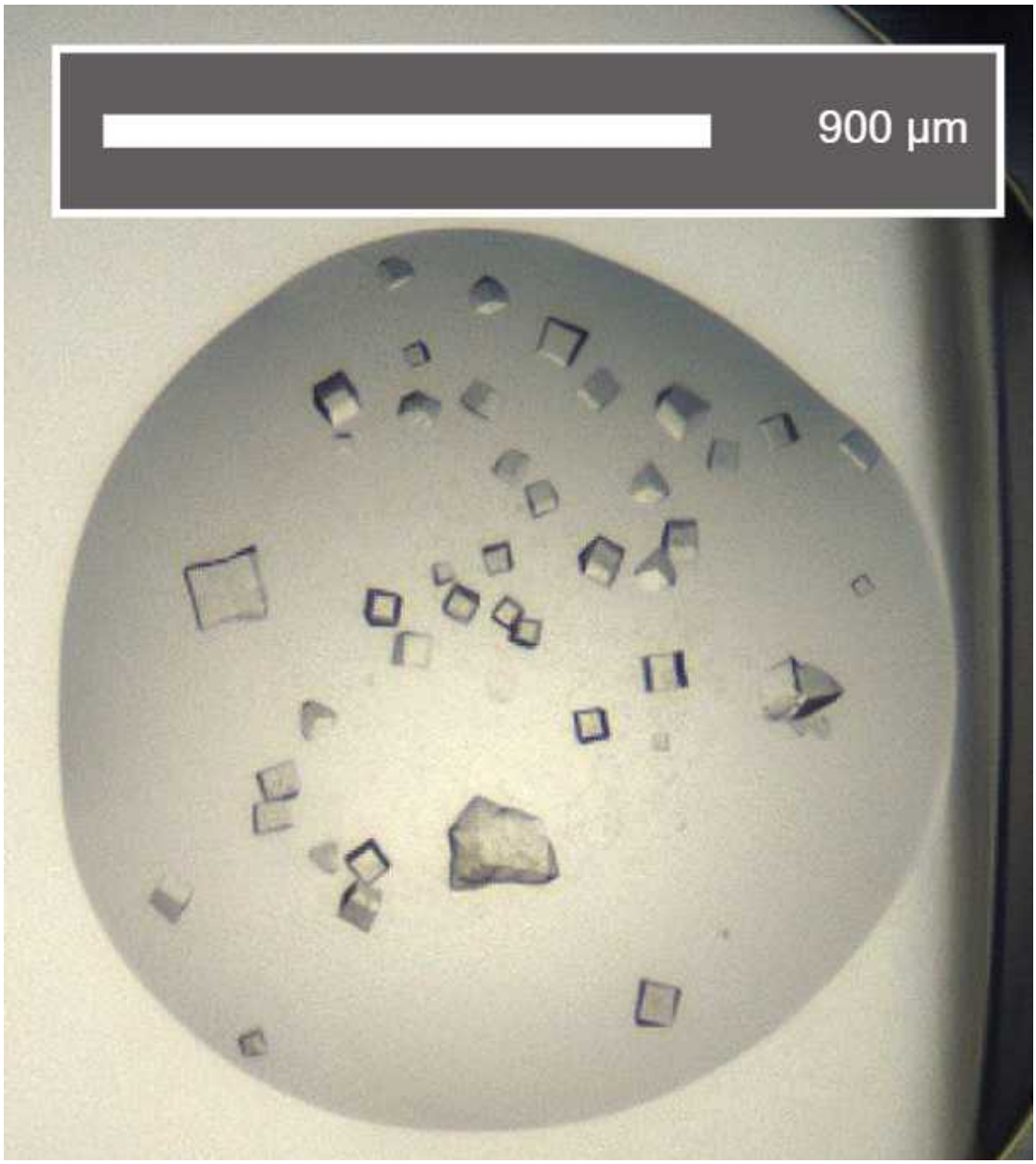
Imaging Schedule: The first images are taken after 12 h and the imaging schedule follows a Fibonacci sequence of days for further collections.

6

Expected result

Crystals typically form within 12 h, within 24 h they have reached their maximum size with slight precipitant. Crystals form on their own and have cubic appearance. (see image below)

Morphology: cubic
Size: ~50 µm in length, width and depth
Average resolution: 1.7 Å
Space group: $P4_322$
Unit cell: 43 Å, 43 Å, 217 Å
90.00°, 90.00°, 90.00°



An example of a drop containing Zika NS2BNS3 crystals.

Data collection at Synchrotron

7 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): 9.50e+11

Protocol references

N/A