

Apr 17, 2025

Version 2

Crystallization of Zika virus NS2B-NS3 protease (closed conformation in space group $P4_322$ | PDB ID: 8PN6) V.2



Version 1 is forked from [ASAP protocol: Crystallization of Enterovirus A71 3C protease \(PDB code 8CNY\)](#)



DOI

<https://dx.doi.org/10.17504/protocols.io.eq2lyj51mlx9/v2>

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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) 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. The crystals appeared within 24 hours and diffracted to 1.7Å in average. The NS2B-NS3 structure is in closed conformation and has been deposited to PDB (PDB code: 8PN6). In this version we added the addgene id and the protein production protocol.



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  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,  7.5 ,

[M] 0.5 Molarity (M) NaCl, 5% glycerol, [M] 0.5 millimolar (mM) TCEP

Protein construct <https://www.addgene.org/204791/>

Protocol materials

 Zika virus portion of NS2B protease coexpressed with NS3 protease **addgene Catalog #228663**

Safety warnings

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



Protein expression and purification

- 1 The protein used for this assay was expressed and purified following the protocol below. The plasmid used was:



Zika virus portion of NS2B protease coexpressed with NS3 protease **addgene Catalog #228663**

Protocol

NAME

Zika NS2B-NS3 protease co-expression construct small scale expression and purification protocol

CREATED BY

korvus.wang

Preview

Equipment needed

- 2 **Formulatrix Rock Imager**
SPT mosquito
Liquidator 96 5-200 µl pipette
SwissCI 3 lens plate

Crystallization experiment

1d

- 3 Detailed information of the construct used for crystallization is in publication: **Zika NS2B-NS3 protease co-expression construct small scale expression and purification protocol**
- 4 **Crystallisation screen composition:**
30% w/v PEG 2000
[M] 0.2 Molarity (M) Ammonium sulfate
[M] 0.1 Molarity (M) sodium acetate  4.8

**Stock solutions used:**

[M] 1 Molarity (M) Ammonium sulfate

[M] 1 Molarity (M) Sodium acetate  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.

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

6 Dispense  30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.

Dispense  150 µL nl [M] 15 mg/mL  Sample to each lens using the SPT mosquito.

Dispense  150 µL Crystallisation screen to each lens using the SPT mosquito.

Drop ratio: 1:1**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

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)

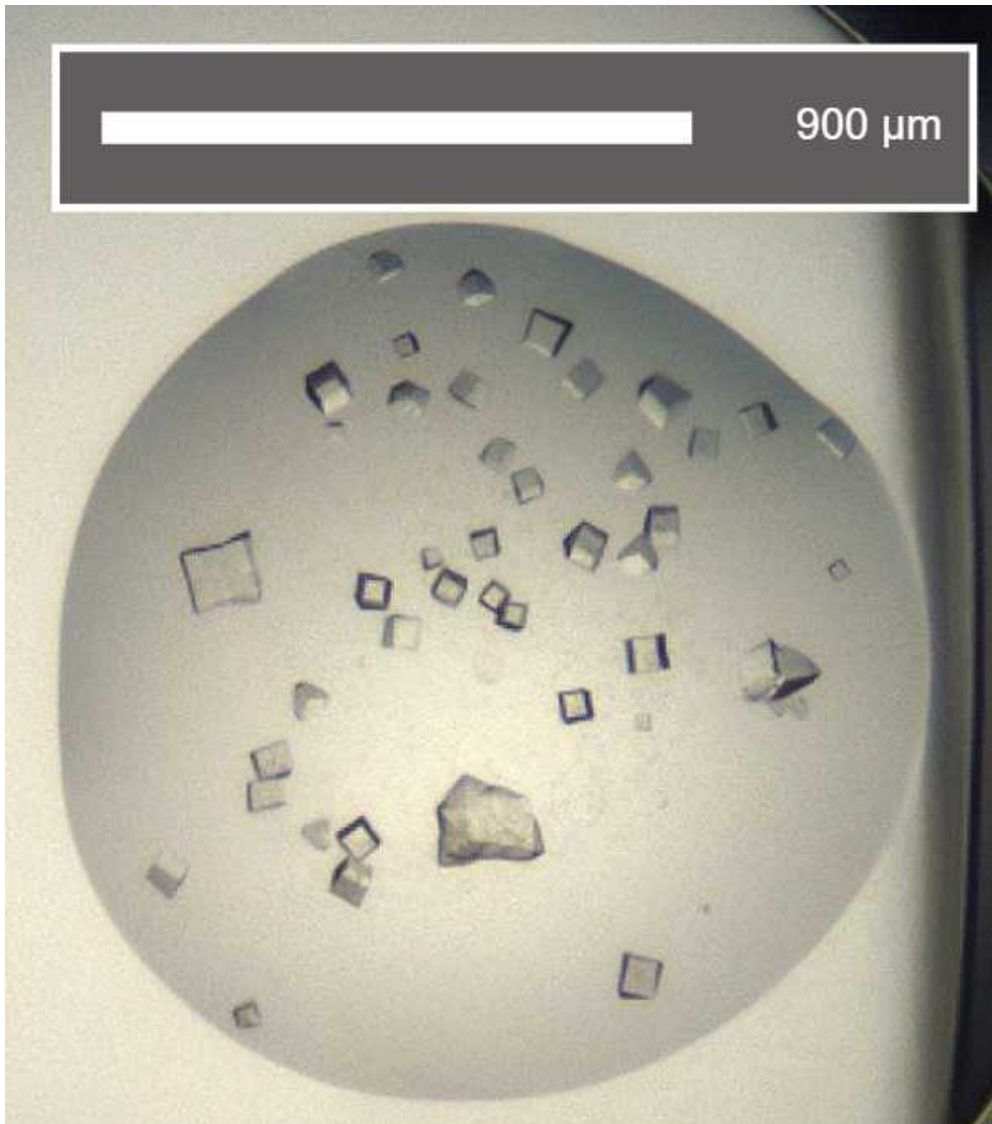
Morphology: cubic

Size: ~50 μm in length, width and depth

Average resolution: 1.7 $\text{\AA}$

Space group: $P4_322$

Unit cell: 43 $\text{\AA}$, 43 $\text{\AA}$, 217 $\text{\AA}$
90.00°, 90.00°, 90.00°



An example of a drop containing Zika NS2B-NS3 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): 9.50e+11

Protocol references

N/A