

Jun 05, 2025

Version 2

🌐 Crystallisation of West Nile virus NS2B-NS3 protease in P6₄ (PDB ID: 8CO8) V.2

🔗 Version 1 is forked from [Crystallisation of SARS-CoV-2 Mpro](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.x54v9293zl3e/v2>

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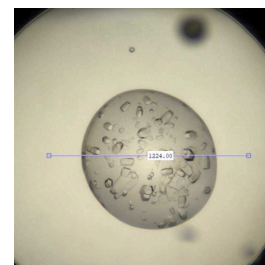
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External link: <https://asapdiscovery.org/outputs/target-enabling-packages/>

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<https://dx.doi.org/10.17504/protocols.io.x54v9293zl3e/v2> Version created by **Mary-Ann Xavier**

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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

West Nile virus NS2B-NS3 innactive fusion protease was crystallized using vapor diffusion in Morpheus screen conditions at pH 8.5. Hexagonal rod-shaped crystals grew to ~50 µm in length after 14 days at 20°C. The crystals belonged to space group P64 and diffracted to 1.91 Å resolution at Diamond Light Source beamline I04. The structure has been deposited as PDB ID 8CO8. In this version, we added the Addgene id of the plasmid used for the protein expresssion and purification.



Materials

Morpheus screen single reagent F12 2-24, Catalog # MDSR-46-250-

Morpheus crystal screen (<https://moleculardimensions.com/en/product/MD1-47>)

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

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

Protocol materials



WNV NS2B-NS3 protease (catalytically active, self cleave); West Nile NS2B-NS3 fusion **addgene** Catalog #204795

Safety warnings



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



Protein expression and purification

3d

- 1 The protein was purified and expressed using the following protocol using an inactive construct of NS2B-NS3. Details in the protocol below.

3d



WNV NS2B-NS3 protease (catalytically active, self cleave); West Nile NS2B-NS3 fusion **addgene Catalog #204795**

Protocol



NAME

West Nile Virus NS2B-NS3 inactive fusion protease expression and purification: large scale 1 L cultures

CREATED BY

Michael Fairhead

[Preview](#)

Equipment needed

- 2 **Formulatrix Rock Imager** (or incubator of choice)
SPT mosquito
Liquidator 96 5-200 μ L pipette (or pipette of choice)
SwissCI 3 lens plate

Crystallisation experiment

1d

- 3 **Protein and buffer requirements:**

30 μ L

[M] 94 mg/mL



Sample

30 μ L Morpheus screen well F12 or tube 2-24

- 4 **Crystallisation screen composition:**



[M] 0.12 Molarity (M)

Monosaccharides



[M] 0.1 Molarity (M) buffer system 3 8.5

37.5 % v/v precipitant mix 4

5 **Drop ratio:** 1:2 (50 nL Sample : 100 nL reservoir solution)

10m

Final drop volume: 150 nL

Dispense 30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using the Liquidator.

Dispense 50 µL [M] 94 mg/mL Sample to each lens using the SPT mosquito.

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

6 Incubate at 20 °C for 336:00:00 hours (14 days) in Formulatrix Rock Imager.

2w

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

7 Crystal first observed and harvested on day 14.

Expected result

Crystals typically form hexagonal rods approximately 50 μm in size.

Morphology: Hexagonal rods.

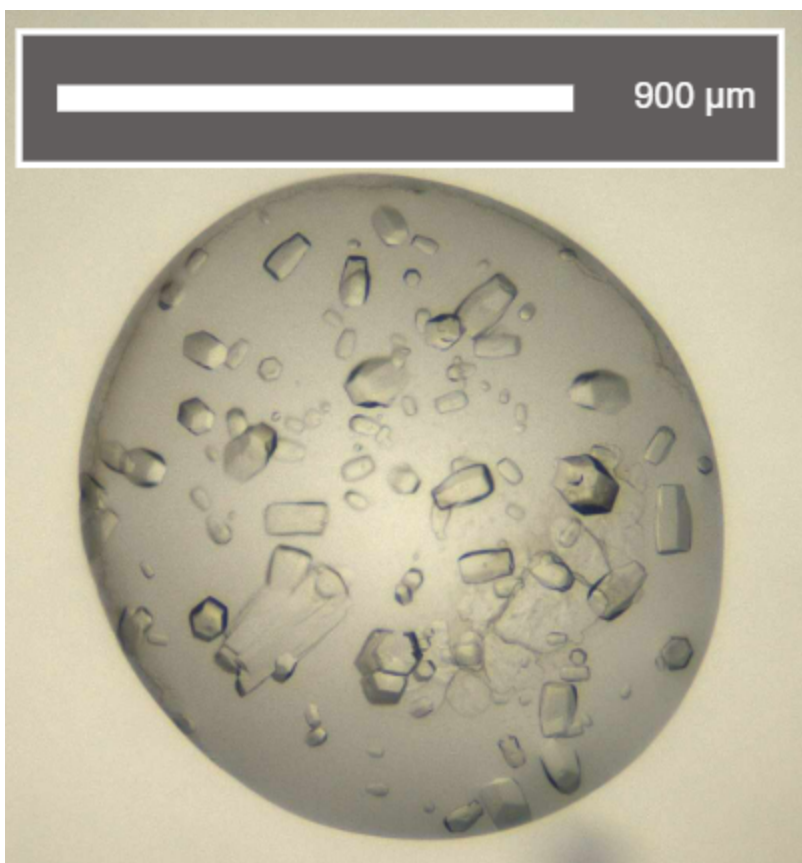
Size: ~50 μm in length, 20 μm in width, 20 μm in depth

Appearance: varied sized of hexagonal rods

Average resolution: 1.9 $\text{\AA}$

Space group: $P6_4$

Unit cell: 56 $\text{\AA}$, 56 $\text{\AA}$, 103 $\text{\AA}$
90.00°, 90.00°, 120.00°



Exemplar picture of West Nile NS2B-NS3 protease crystals.

Data collection at Synchrotron



8 Diamond Light Source
Unattended Data Collection (UDC)
Data Collection Temperature: 100K
Detector: DECTRIS EIGER2 X 9M
Beamline: I04
Wavelength: 0.9537 Å
Resolution (Å): 1.91
Beam Size (µm): 30 X 20
Number of images: 3600
Oscillation: 0.10°
Exposure (s): 0.0053
Transmission (%): 100
Flux (ph/s): 9.50e+11

Protocol references

N/A