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Version 1

Crystallisation of West Nile virus NS2B-NS3 protease in P6₄ (PDB ID: 8CO8) V.1



Forked from [Crystallisation of SARS-CoV-2 Mpro](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.x54v9293zl3e/v1>

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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 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.



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

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

Protocol



NAME

West Nile Virus NS2B-NS3 protease fusion protein 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 µl pipette (or pipette of choice)
SwissCI 3 lens plate

Crystallisation experiment

1d

- 3 **Protein and buffer requirements:**
 30 µL 94 mg/mL Sample
 30 µL Morpheus screen well F12 or tube 2-24
- 4 **Crystallisation screen composition:**
 0.12 Molarity (M) Monosaccharides
 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)
Final drop volume: 150 nL

10m



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

Dispense  50 nL  94 mg/mL  Sample to each lens using the SPT mosquito.

Dispense  100 nL 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.

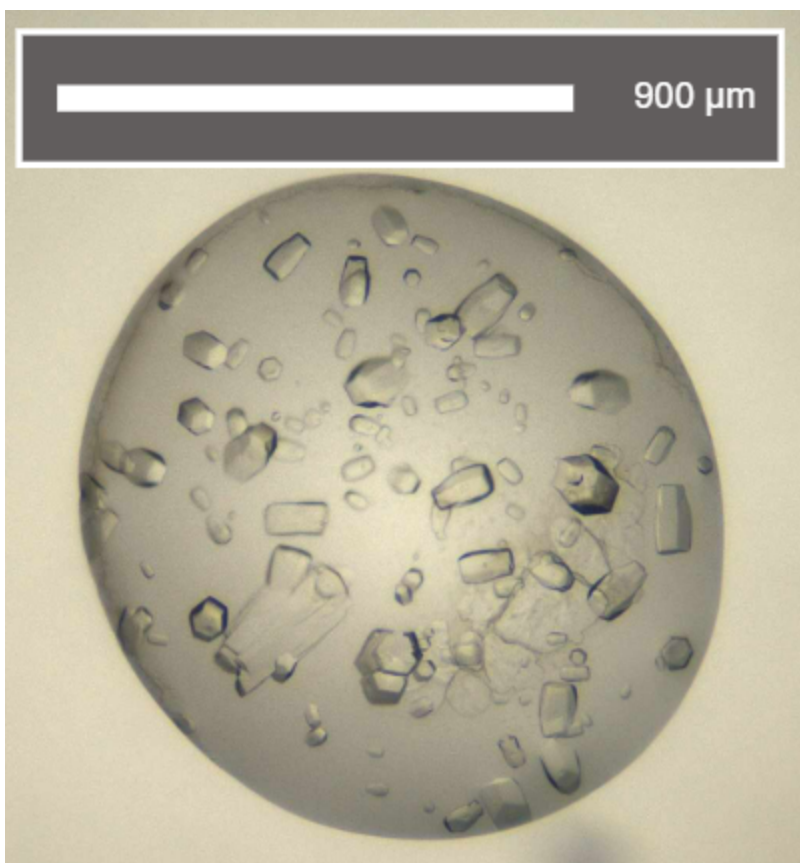
Size: ~50 μm in length, 20 μm in width, 20 μm in depth

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