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Crystallisation of Zika NS5 RdRp

Forked from a private protocol

DOI

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ASAP Discovery



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External link: <https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-ZIKA-NS5-RDRP>

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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 main aim of this work was to identify small molecules that bind Zika NS5 RdRp (catalytic RNA-dependent RNA polymerase domain) through X-ray fragment-based screening. The Zika NS5 RDRP domain was cloned, expressed, purified, and crystallised. Suitable crystals for fragment screening were produced and optimised allowing an extensive fragment campaign to be performed. A native high-resolution structure was determined at 1.8Å and formed the basis for the fragment campaign.



Materials

SwissCI 3 lens crystallization plates <https://swissci.com/product/3-lens-crystallisation-plate/> Codes:
Midi: UVXPO-3LENS 3W96T-PS 3W96T-UVP

Morpheus HT-96 single reagent 250mL Catalog # MDSR-47-250-2-10

Purified Zika NS5 polymerase protein ([M] 5 mg/mL) in [M] 20 millimolar (mM) HEPES  7.5 ,
[M] 300 millimolar (mM) NaCl, 2.5% Glycerol, [M] 10 micromolar (μ M) ZnCl_2 , [M] 2 millimolar (mM) TCEP

306-903 residues- construct 2A1 (6 Hist sumo tag)

Safety warnings

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

Zika NS5 RdRp expression and purification

- 1 The protein used for crystallisation was expressed and purified using the following protocol.

Protocol

NAME

Zika NS5 RdRp His-SUMO construct small scale expression and purification protocol

CREATED BY

korvus.wang

Preview

Equipment needed

- 2 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

Crystallisation experiment

1d

**3 Protein and buffer requirements:**

28.8 µL 5 mg/mL Sample

3.264 mL Crystallisation screen

4 Crystallisation screen composition:

Morpheus I E10 condition

0.12 Molarity (M) Ethylene glycols

0.1 Molarity (M) Buffer system 3 8.5

30 % v/v Precipitant Mix 2

Stock solutions used:

Morpheus I E10 condition

Note

The crystallisation screen can be stored in a duran bottle.

For long term storage keep the crystallisation screen in the fridge at 4°C.

5 Dispense 34 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.

Dispense 100 µL 5 mg/mL Sample to each lens using the SPT mosquito.

Dispense 50 µL Crystallisation screen to each lens using the SPT mosquito.

Drop ratio: 2:1 ratio (100 nl Sample : 50 nl reservoir solution)

Final drop volume: 150 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 Crystal form after ~24 h.

Expected result

The crystals reach their maximum size after 96 h and the precipitant has gone.

Crystals grown inconsistently to 2 sizes, one being half the size described below, but both sizes achieve the same results

Morphology: typically plates.

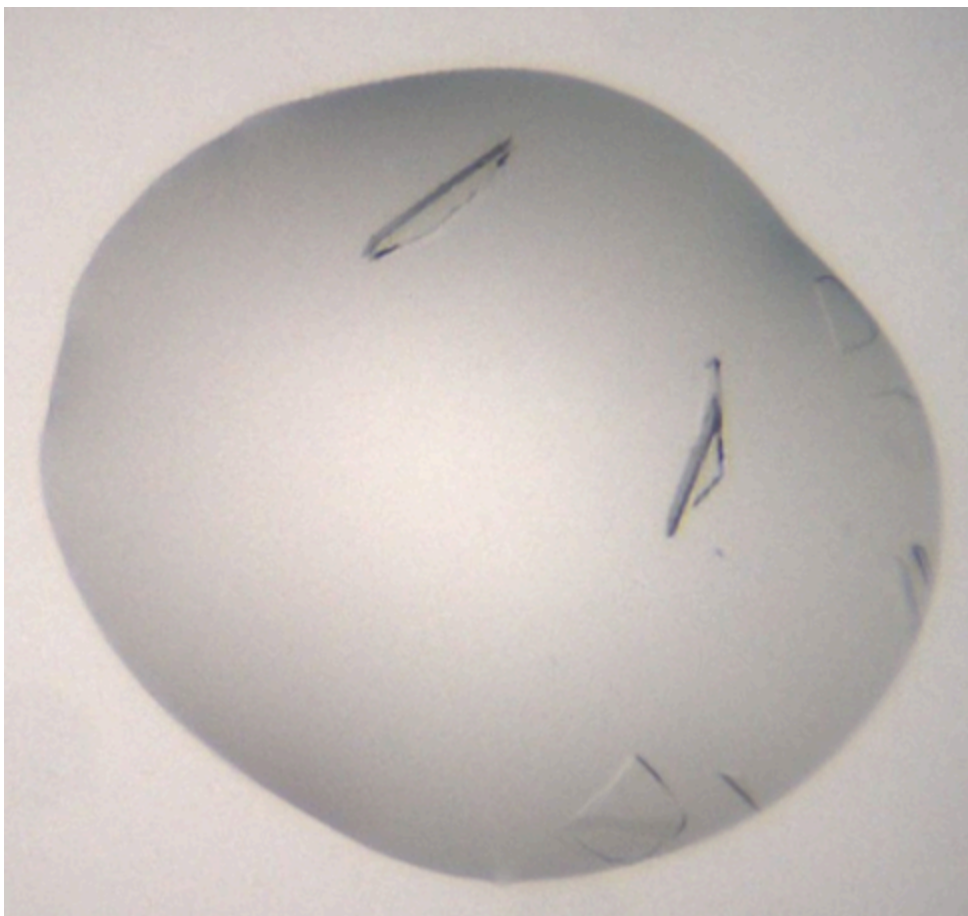
Size: ~ 250 μm in length and ~60 μm in width, depth of the crystals is ~2 μm

Appearance: glass shard.

Average resolution: 2.0 $\text{\AA}$

Space group: $P4_32_12$

Unit cell: 79 $\text{\AA}$, 79 $\text{\AA}$, 210 $\text{\AA}$
90.00°, 90.00°, 90.00°



An example of a drop containing Zika NS5 RdRp polymerase 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): 1.78
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