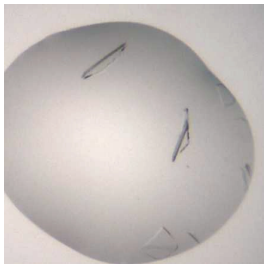
Apr 26, 2024

Crystallization of Zika NS5 RdRp



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[1]Research Complex at Harwell; [2]Diamond Light Source

anu.chandran: The principle crystallographer for the Zika NS5 RdRp polymerase project.;

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 pH 7.5 , [M] 300 millimolar (mM) NaCl, 2.5% Glycerol, [M] 10 micromolar (µM) $ZnCl_2$, [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 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:**
28.8 µL | [M] 5 mg/mL | Sample
3.264 mL Crystallization screen

3 **Crystallisation screen composition:**
Morpheous I E10 condition

[M] 0.12 Molarity (M) Ethylene glycols
[M] 0.1 Molarity (M) Buffer system 3 pH 8.5
30 % v/v Precipitant Mix 2
Stock solutions used:
Morpheous 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.

4 Dispense 34 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.
Dispense 100 undetermined [M] 5 mg/mL Sample to each lens using the SPT mosquito.
Dispense 50 undetermined 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

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

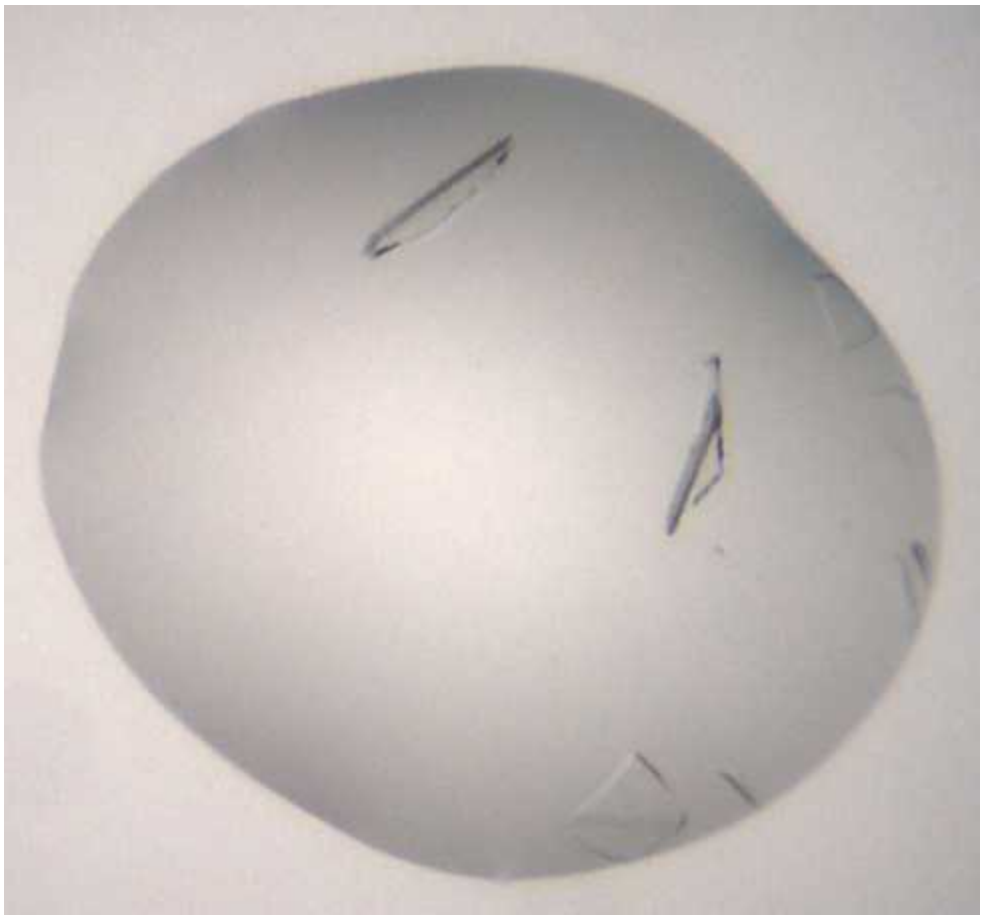
Imaging Schedule: The first images are taken after 12 h and the imaging schedule follows a Fibonacci sequence of days for further collections.

6 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 Å
Space group: $P4_32_12$
Unit cell: 79 Å, 79 Å, 210 Å
90.00°, 90.00°, 90.00°



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