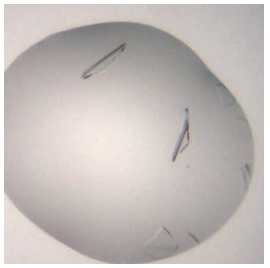
Oct 02, 2024

Crystallisation of Zika NS5 RdRp



Forked from Crystallisation of Zika NS5 RdRp

DOI
dx.doi.org/10.17504/protocols.io.5jyl82746l2w/v1

Anu V. Chandran[1], Peter Marples[1,2], Martin austin Walsh[1]

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

Anu V. Chandran: The principle crystallographer for the Zika NS5 RdRp polymerase project.;

ASAP Discovery

Peter Marples
Diamond Light Source

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5jyl82746l2w/v1

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

Protocol Citation: Anu V. Chandran, Peter Marples, Martin austin Walsh 2024. Crystallisation of Zika NS5 RdRp. **protocols.io https://dx.doi.org/10.17504/protocols.io.5jyl82746l2w/v1**

Protocol status: Working
We use this protocol and it's working

Created: October 02, 2024

Last Modified: October 02, 2024

Protocol Integer ID: 108828

Keywords: crystallisation, XChem, ASAP, AViDD, CMD, Diamond Light Source, i04-1, Zika NS5 RdRp, NS5 RdRp, Research complex at Harwell

Funders Acknowledgement:
National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAID)
Grant ID: Grant ID: U19AI171399

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgements:

Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0QX, UK
Research Complex at Harwell, Harwell Science and Innovation Campus, Didcot OX11 0FA, UK
Oxford Lab Technologies crystal shifter https://doi.org/10.1107/S2059798320014114

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.

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 [M] 5 mg/mL Sample
3.264 mL Crystallisation screen

4 **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.

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

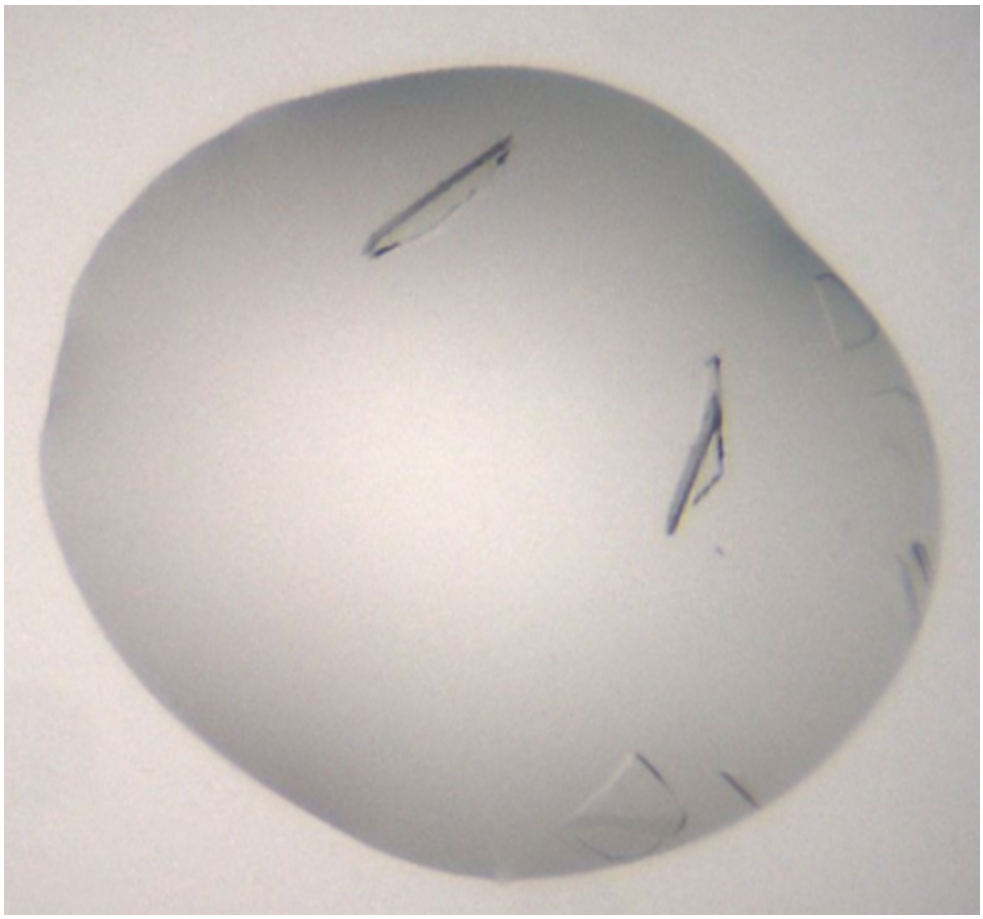
Imaging Schedule: The first images are taken after 12 h and the imaging schedule follows a Fibonacci sequence of days for further collections.

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 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

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