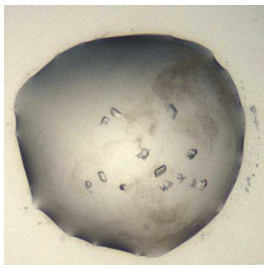
Apr 26, 2024

Crystallization of Enterovirus D68 3C protease



DOI
dx.doi.org/10.17504/protocols.io.5qpvoky29l4o/v1

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ryan Lithgo: The principle crystallographer on the Enterovirus 3C protease ProB project.;

ASAP Discovery

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OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.5qpvoky29l4o/v1

Protocol Citation: ryan Lithgo, Peter Marples, Lizbé Koekemoer, Daren Fearon 2024. Crystallization of Enterovirus D68 3C protease. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvoky29l4o/v1

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

Created: April 26, 2024

Last Modified: April 26, 2024

Protocol Integer ID: 98840

Keywords: crystallisation, 3C protease, XChem, ASAP, AViDD, CMD, Diamond Light Source, i04-1, D68 3C protease

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 development of effective broad-spectrum antivirals forms an important part of preparing for future pandemics. A cause for concern is the currently emerging pathogen Enterovirus D68 (EV-D68) which primarily spreads through respiratory routes causing mostly mild to severe respiratory illness but, in severe cases, acute flaccid myelitis. The 3C protease of EV-D68 is a potential target for the development of antiviral drugs due to its essential role in the viral life cycle and high sequence conservation. This protocol was used to grow D68 3C ProB crystals that were applied high-throughput crystallographic follow up compound screening on D68 3C.

Materials

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

[M] 1 Molarity (M) Tris adjusted to pH 7.8 with NaOH, Molecular Dimensions, Catalog # MD2-027-PH 7.8
[M] 1 Molarity (M) Ammonium acetate, Molecular Dimensions, Catalog # MD2-002-PH
50% w/v PEG 3350, Molecular Dimensions, Catalog # MD2-250-9

Purified D683C protein ([M] 35 mg/mL) in [M] 10 millimolar (mM) HEPES, pH 7.5 , [M] 0.5 Molarity (M) NaCl, 5% glycerol, [M] 0.5 millimolar (mM) TCEP

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 **Prepare seed stock:**

Protocol	
NAME	**Diamond XChem Seeding Protocol**
CREATED BY	**Peter Marples**
PREVIEW	

1: 1 000 000 dilution Sample seeds

3 **Protein and buffer requirements:**

14.4 µL [M] 35 mg/mL Sample
2.88 mL Crystallization screen

7.2 µL seeds, dilution 1:1 000 000

4 **Crystallisation screen composition:**

[M] 0.1 Molarity (M) Tris pH 7.8

[M] 0.2 Molarity (M) Ammonium acetate

26% w/v PEG 3350

Stock solutions used:

[M] 1 Molarity (M) Tris adjusted to pH 7.8 with NaOH

[M] 1 Molarity (M) Ammonium acetate

50% w/v PEG 3350

Note

The crystallisation screen can be stored in a duran bottle or aliquoted into 96 deep well block for easy dispensing into SwissCI 3 lens plates.

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

5 Dispense 30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.

Dispense 50 undetermined [M] 35 mg/mL Sample to each lens using the SPT mosquito.

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

Dispense 25 undetermined Seeds to each lens using the SPT mosquito.

Drop ratio: 2:4:1

Final drop volume: 175 nl

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

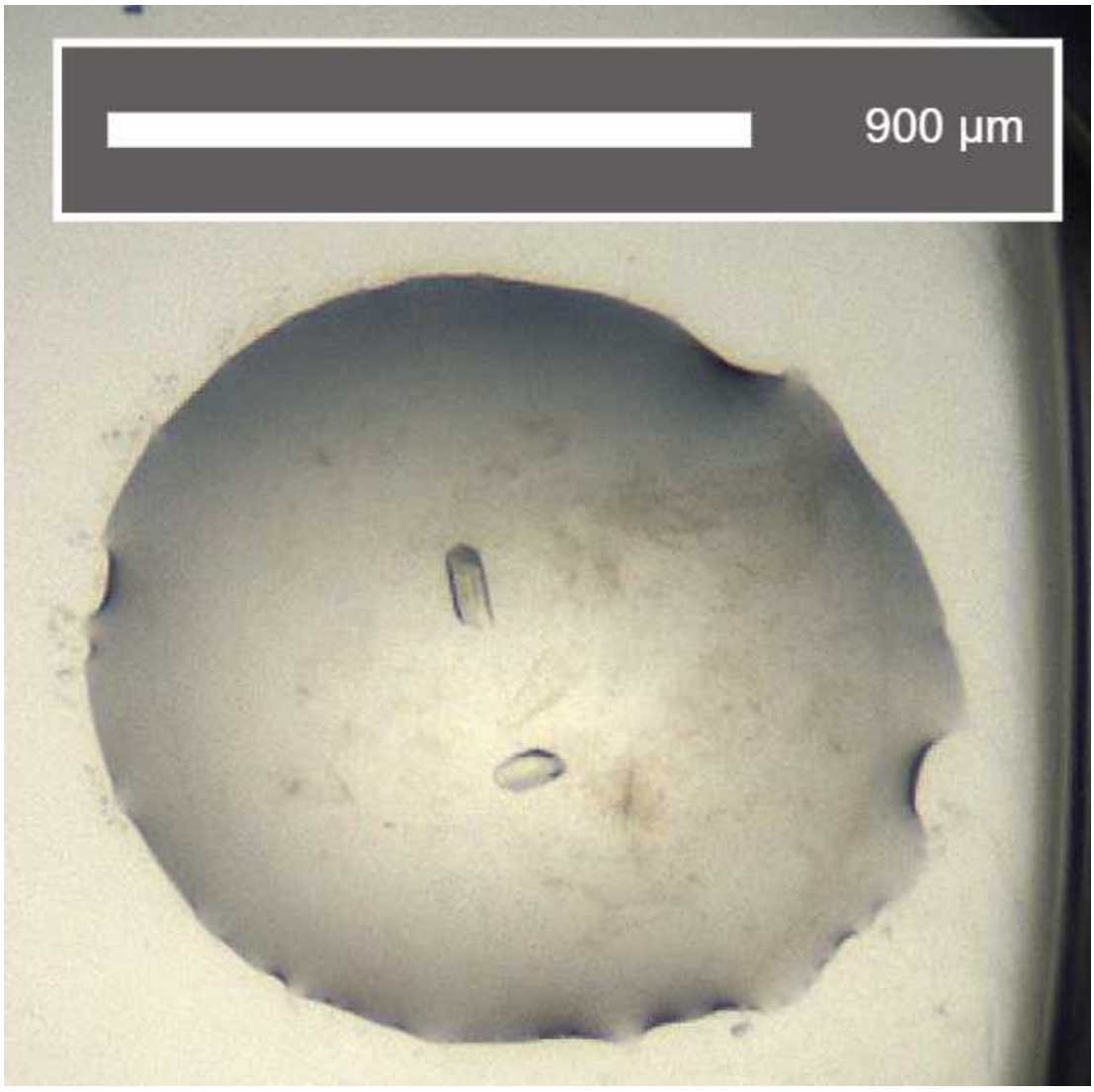
Imaging Schedule: The first images are taken after 12 h and the imaging schedule follows a Fibonacci sequence of days for further collections.

7

Expected result

Crystals typically appear after 24 hours and reach their maximum size after ~24 h with some precipitation often remaining.

Morphology: small shards.
Size: ~40 µm in length and ~40 µm in width, depth of the crystals is ~20 µm, giving a glass shard appearance
Average resolution: 1.5 Å
Space group: $P2_1$
Unit cell: 39.7 Å, 105 Å, 43.5 Å
90.00°, 110.00°, 90.00°



An example of a drop containing D68 3C protease crystals.

Data collection at Synchrotron

8 Diamond Light Source
Unattended Data Collection (UDC)
Data Collection Temperature: 100K
Detector: DECTRIS EIGER2 X 9M
Beamline: I04-1
Wavelength: 0.9212 Å
Resolution (Å): 1.62
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