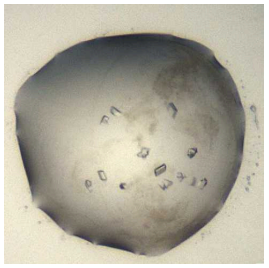
Apr 25, 2025 Version 2

Crystallisation of Enterovirus D68 3C protease in space group $P2_1$ used for follow up compounds V.2



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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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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 current cause for concern is the emerging pathogen Enterovirus D68 (EV-D68), which primarily spreads through respiratory routes. While it mostly causes mild to severe respiratory illness, in severe cases it can lead to acute flaccid myelitis. The 3C protease of EV-D68 is a potential target for antiviral drug development due to its essential role in the viral life cycle and high sequence conservation. This protocol was used to grow EV-D68 3C crystals that were subjected to high-throughput fragment screening crystallography (PDB group deposition G_10002271). In this new version, we have added the protocols for protein expression and purification, soaking conditions, and fragment screening information, as well as the affiliation with the ASAP Discovery Consortium.

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

Protocol materials

Enterovirus D68 Strain STL 2010 12 3C protease **addgene Catalog #**228644

Safety warnings

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

Protein expression and purification protocol

1 The protein used for crystallography used the following protocol for expression and purification.

Enterovirus D68 Strain STL 2010 12 3C protease **addgene Catalog** #228644

Protocol

NAME

Small-scale Expression and Purification Protocol for His-SUMO Tagged Enterovirus D68 Strain STL 2014 12 3C Protease in E. coli

CREATED BY

korvus.wang

Preview

Equipment needed

2 **Formulatrix Rock Imager** (or incubator of choice)
SPT mosquito

P100 8 multi-channel pipette
SwissCI 3 lens plate

Crystallization experiment

1d

3 **Prepare seed stock:**

Protocol

NAME

Diamond XChem Seeding Protocol

CREATED BY

Peter Marples

Preview

1: 1 000 000 dilution Sample seeds

4 **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

5 **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.

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

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

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

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

Drop ratio: 2:4:1
Final drop volume: 175 nl

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

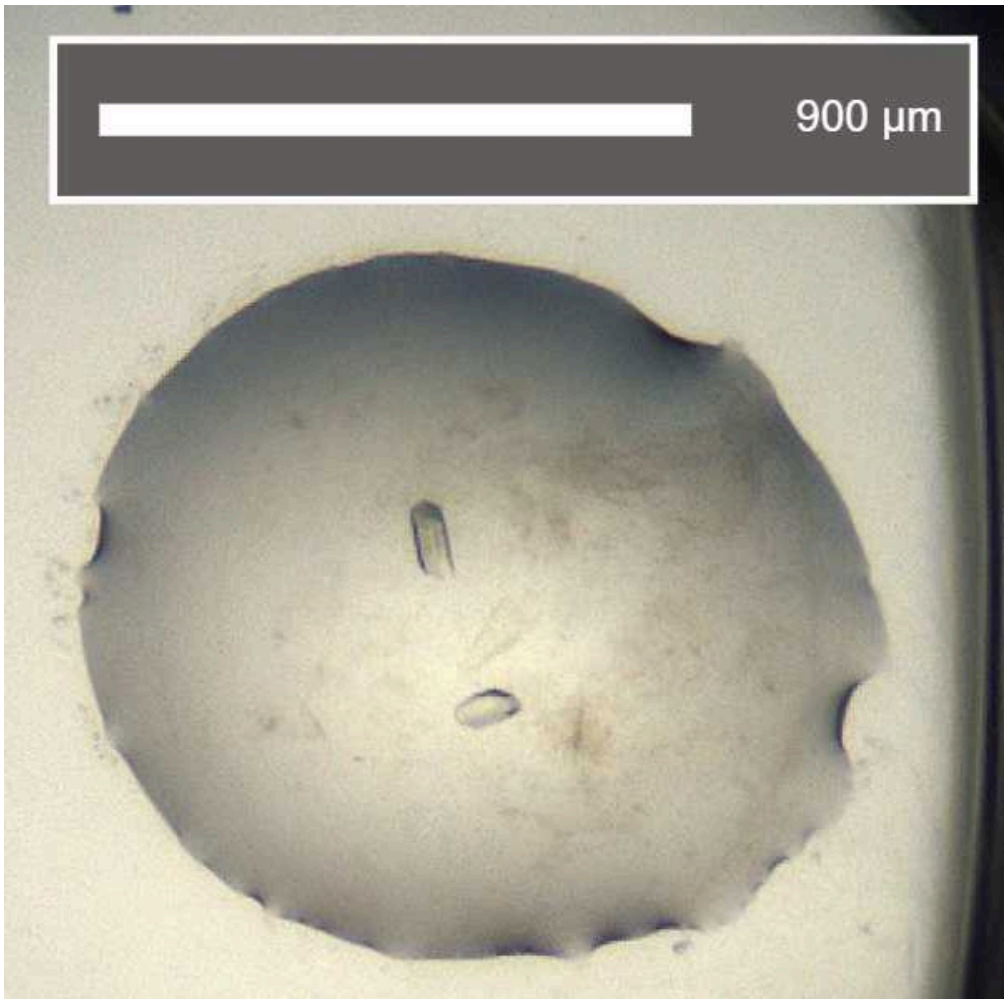
Imaging Schedule: The first images are taken after 12 h and the imaging schedule follows a Fibonacci sequence of days for further collections.

8

Expected result

EEV-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 EV-D68 3C protease crystals.

Data collection at Synchrotron

9 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

Soaking Tolerance

2h 30m

10 Final condition idenitified: 20% DMSO 02:30:00

2h 30m

Protocol

NAME

XChem solvent test

CREATED BY

Peter Marples

Preview

Compound Soaking

2h 30m

11 Condition used: 20% compound, 02:30:00

2h 30m

Protocol

NAME

XChem crystallographic fragment screening

CREATED BY

Peter Marples

Preview

Protocol references

https://www.rcsb.org/groups/summary/entry/G_1002271
https://doi.org/10.1101/2024.04.29.591650