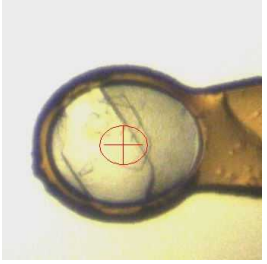
Apr 25, 2025 Version 2

Crystallisation of Enterovirus D68 3C protease apo structure and fragment screen in space group $P2_12_12_1$ (PDB code 8CNX) V.2



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

ASAP Discovery

Lizbé Koekemoer
University of Oxford

DOI: https://dx.doi.org/10.17504/protocols.io.n92ldm6jnl5b/v2

External link: https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-EVD68-3CLPRO

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

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Protocol Integer ID: 119833

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Abstract

The crystallization protocol and buffer conditions used to obtain Enterovirus D68 3C protease crystals. The crystal structure was depostited to RCSB PDB with code **8CNX**.
In this new version we added the Addgene ID and the protein expression and purification protocol. This construct gave us a crystal form suitable for fragment screen and initial compound soaking.

Materials

EV-D68 3C protease; aliases: Enterovirus A71 3C protease, non cleavable C-term 6xHis tag **addgene Catalog #**(Plasmid #204817)

SwissCI 3 lens crystallization plates **https://swissci.com/product/3-lens-crystallisation-plate/**
1 M Tris-HCl pH 8.14
1 M Ammonium acetate
50% w/v PEG 3350

Protocol materials

EV-D68 3C protease; aliases: Enterovirus A71 3C protease, non cleavable C-term 6xHis tag **addgene Catalog #**(Plasmid #204817)

Protein expression and purification

2d

1 Small scale protein purification and expression protocols used for this assay

EV-D68 3C protease; aliases: Enterovirus A71 3C protease, non cleavable C-term 6xHis tag **addgene Catalog #**(Plasmid #204817)

Protocol

NAME

Enterovirus D68 3C protease small scale expression and purification protocol

CREATED BY

korvus.wang

Preview

Equipment needed

2 **Formulatrix Rock Imager** (or incubator of choice)
SPT mosquito
P100 multi-channel pipette
SwissCI 3 lens plate

Crystallisation experiment

1d

3 **Protein and buffer requirements:**

32 µL [M] 1 millimolar (mM) Sample
2.88 mL Crystallization screen
16 µL Sample seeds, dilution 1:100 000

4 **Crystallisation screen composition:**
0.1 M Tris-HCl (pH 8.14)

0.2 M Ammonium acetate
25% w/v PEG 3350

Stock solutions used:
1 M Tris-HCl adjusted to pH 8.14 with HCl
1 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 **Prepare seed stock:**
1: 100 000 dilution Sample seeds

6 Dispense 30 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.
Dispense 100 µL [M] 1 millimolar (mM) Sample to each lens using the SPT mosquito.
Dispense 200 µL Crystallisation screen to each lens using the SPT mosquito.
Dispense 50 µL Seeds to each lens using the SPT mosquito.

Drop ratio: 1:2:05 ratio (100 nl Sample : 200 nl reservoir solution: 50 nl seeds)
Final drop volume: 350 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.

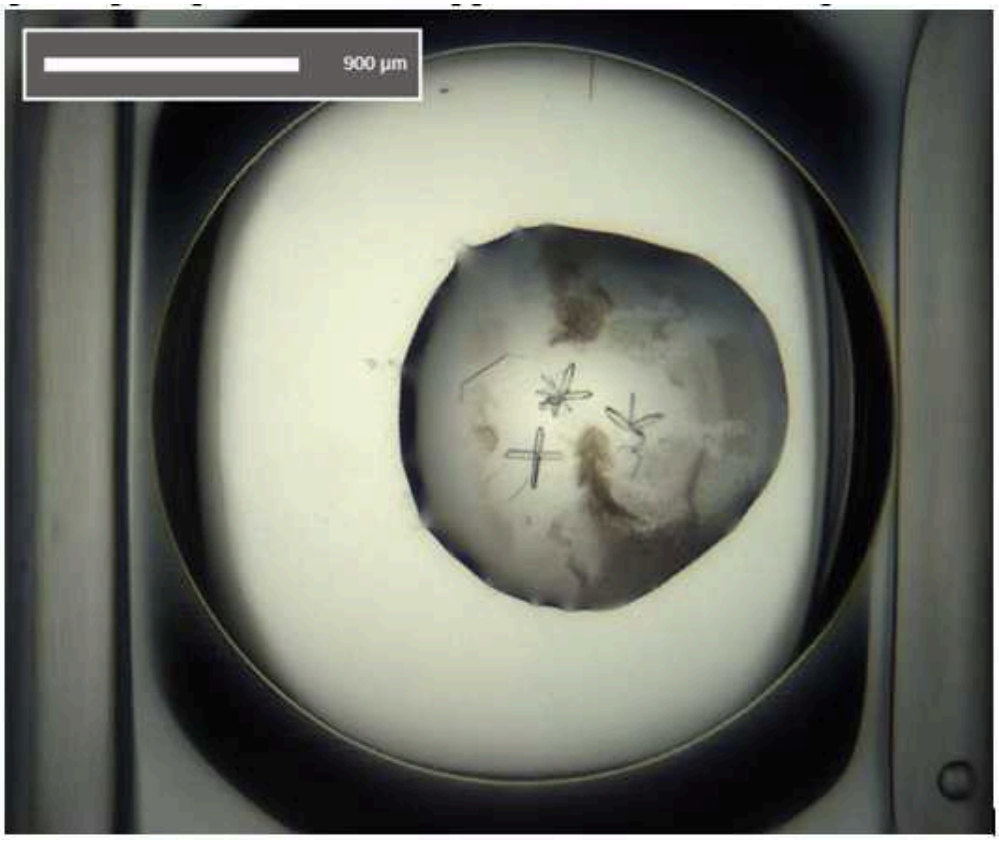
8 Crystal form after ~12 h.

Expected result

The crystals reach their maximum size after 24 h and most of the precipitant has gone.

Crystals typically form either as single crystals or in small clusters containng 4-6 crystals in a flower-like arrangement. Upon mounting a gentle nudge dissociates the cluster.

Morphology: typically thin rectangles with pointed ends.
Size: ~100 µm in length and ~50 µm in width, depth of the crystals is ~10 µm
Appearance: glass shard.
Average resolution: 1.5 Å
Space group: $P2_12_12_1$
Unit cell: 42.82 Å, 62.53 Å, 147.36 Å
90.00°, 90.00°, 90.00°



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

Soaking tolerance

2h 30m

9 Final condition idenitified: 20% DMSO, 2hr 30mins using the protocol bellow. 2h 30m

Protocol

NAME

XChem solvent test

CREATED BY

Peter Marples

Preview

Fragment and compound soaking 2h 30m

10 20% fragment/compound, 2hr 30mins using the protocol bellow. 2h 30m

Protocol

NAME

XChem crystallographic fragment screening

CREATED BY

Peter Marples

Preview

Protocol references

N/A