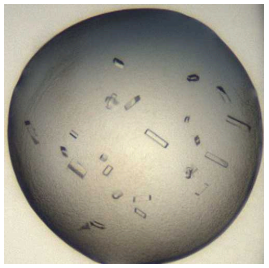
Apr 25, 2025 Version 2

Crystallisation of Enterovirus coxsackievirus A16 2A protease in space group C2 used for apo, fragment screen and follow compounds (PDB 8POA) 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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Abstract

Picornaviridae coxsackievirus A16 is the causative agent of paediatric hand-foot-and-mouth disease, and a target for pandemic preparedness due to the risk of higher order complications in a large-scale outbreak. The 2A protease of the virus is responsible for self-cleavage from the poly protein, allowing for correct folding and assembly of capsid proteins in the final stages of viral replication. Inhibition deranges capsid folding and assembly, preventing formation of mature virions in host cells and making the protease a valuable target for antiviral activity. This protocol was used to grow coxsackievirus A16 crystals (PDB 8POA) that were used in high-throughput crystallographic fragment screening, and follow up compounds on the target. In this new version we added: the group deposition code; details about the fragment screen and solvent tolerance; also the protein production protocol.

Materials

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

[M] 1 Molarity (M) MES pH 6.7, Molecular Dimensions, Catalog # MD2-013-PH 6.7
50% w/v PEG 20000, Molecular Dimensions, Catalog # MD2-250-16

Purified SARS CoV-2 Coxsackievirus A16 protein ([M] 20 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

Protein construct **https://www.addgene.org/204809/**

Protocol materials

Human Coxsackievirus A16 strain G10 2A protease **addgene Catalog #**228632

Safety warnings

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

Protein expression and purification

1 We used the following protein expression and purification protocol for the plasmid
Human Coxsackievirus A16 strain G10 2A protease **addgene Catalog #**228632

Protocol

NAME

Enterovirus coxsackievirus A16 2A 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 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: 1000 dilution Sample seeds

4 **Protein and buffer requirements:**

43.2 µL [M] 20 mg/mL Sample

3.36 mL Crystallization screen

14.4 µL Sample seeds, dilution 1:1000

5 **Crystallisation screen composition:**

13.5 % PEG 20000

[M] 0.1 Molarity (M) MES pH 6.7

Stock solutions used:

[M] 1 Molarity (M) MES pH 6.7

50% w/v PEG 20000

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

Dispense 150 µL [M] 20 mg/mL Sample to each lens using the SPT mosquito.

Dispense 150 µL Crystallisation screen to each lens using the SPT mosquito.

Dispense 50 µL Seeds to each lens using the SPT mosquito.

Drop ratio: 3:3:1 ratio (150 nl Sample : 150 nl reservoir solution: 50 nl seeds)

Final drop volume: 350 nl

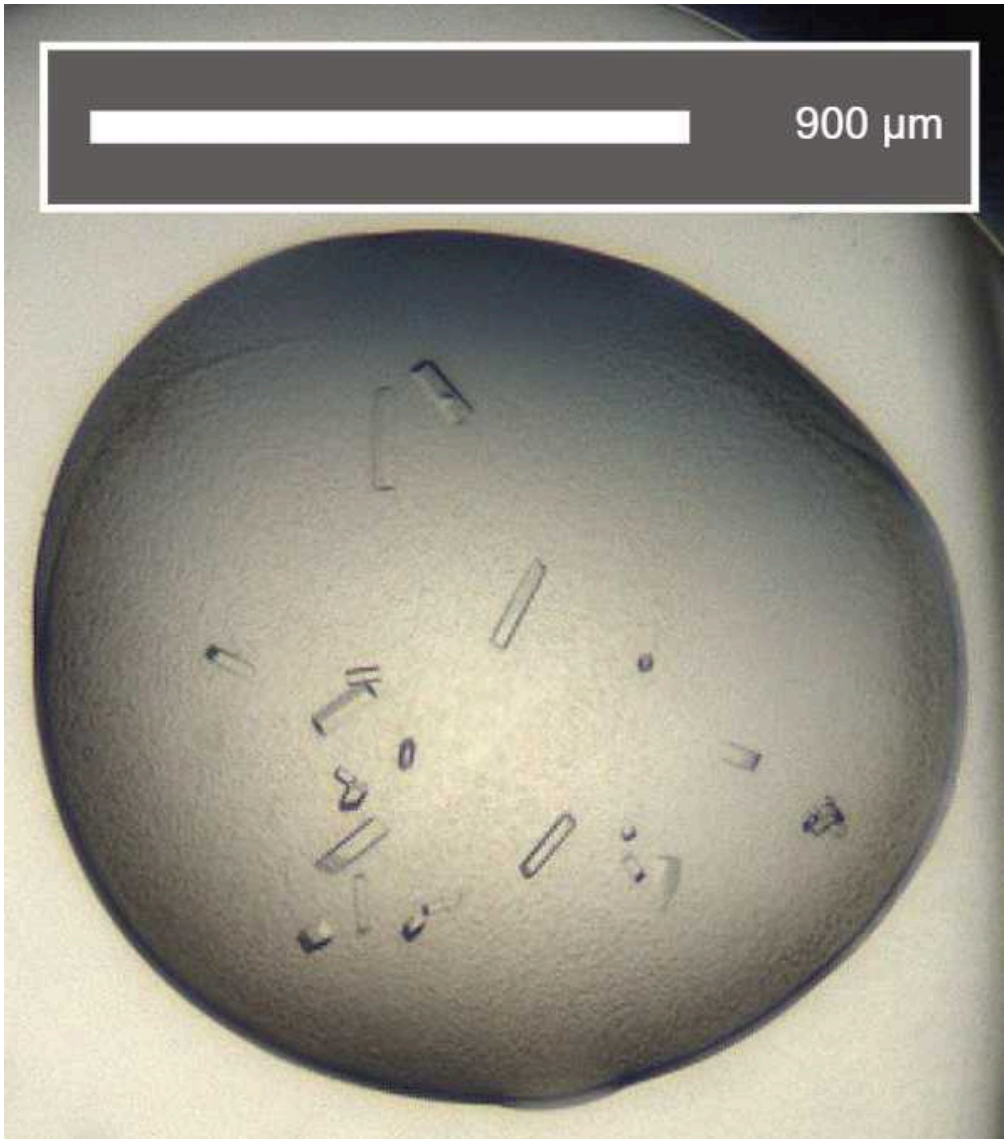
7 Incubate at 20 °C for 24:00:00 h in Formulatrix Rock Imager. 1d

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

8 Crystal typically form after ~24hrs

Expected result

Crystals typically reach their maximum size after ~24 h.
Morphology: typically rectangles.
Size: ~75 µm in length and ~10 µm in width, depth of the crystals is ~10 µm, giving a rectangular appearance
Average resolution: 1.6 Å
Space group: C2
Unit cell: 86 Å, 57 Å, 32 Å
90°, 95°, 90°



An example of a drop containing Coxsackievirus A16 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): 1.21
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

Solvent tolerance

10 Best conditions were: 10%DMSO, for 1h.

Protocol

NAME

XChem solvent test

CREATED BY

Peter Marples

Preview

Fragment and compound soaking

11 Conditions used 5-10% DMSO, for 1h

Protocol

NAME

XChem crystallographic fragment screening

CREATED BY

Peter Marples

Preview

Results

12 The results of this fragment screen was deposited under the PDB code G_1002288
https://www.rcsb.org/groups/summary/entry/G_1002288

Protocol references

https://www.rcsb.org/groups/summary/entry/G_1002288

https://www.rcsb.org/structure/8POA

https://doi.org/10.1101/2024.04.29.591684