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🌐 Crystallisation of Enterovirus coxsackievirus A16 2A protease



Forked from [Crystallization of Enterovirus coxsackievirus A16 2A protease](#)

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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 that were applied high-throughput crystallographic fragment screening on the target.



Materials

<https://swissci.com/product/3-lens-crystallisation-plate/> Codes:

Midi: UVXPO-3LENS 3W96T-PS 3W96T-UVP

[M] 1 Molarity (M) MES  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,  7.5 ,

[M] 0.5 Molarity (M) NaCl, 5% glycerol, [M] 0.5 millimolar (mM) TCEP

Protein construct <https://www.addgene.org/204809/>

Safety warnings

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



Enterovirus coxsackievirus A16 2A protease expression and purification

- 1 The protein used for crystallisation was expressed and purified using the following protocol.

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

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 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 μ L [M] 20 mg/mL Sample

3.36 mL Crystallization screen

 14.4 μ L Sample seeds, dilution 1:1000

5 Crystallisation screen composition:

13.5 % w/v PEG 20000

[M] 0.1 Molarity (M) MES 6.7

Stock solutions used:

[M] 1 Molarity (M) MES 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 μ L Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 μ l multi-channel pipette.

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

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



Dispense  50 nL 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 ~36 h.

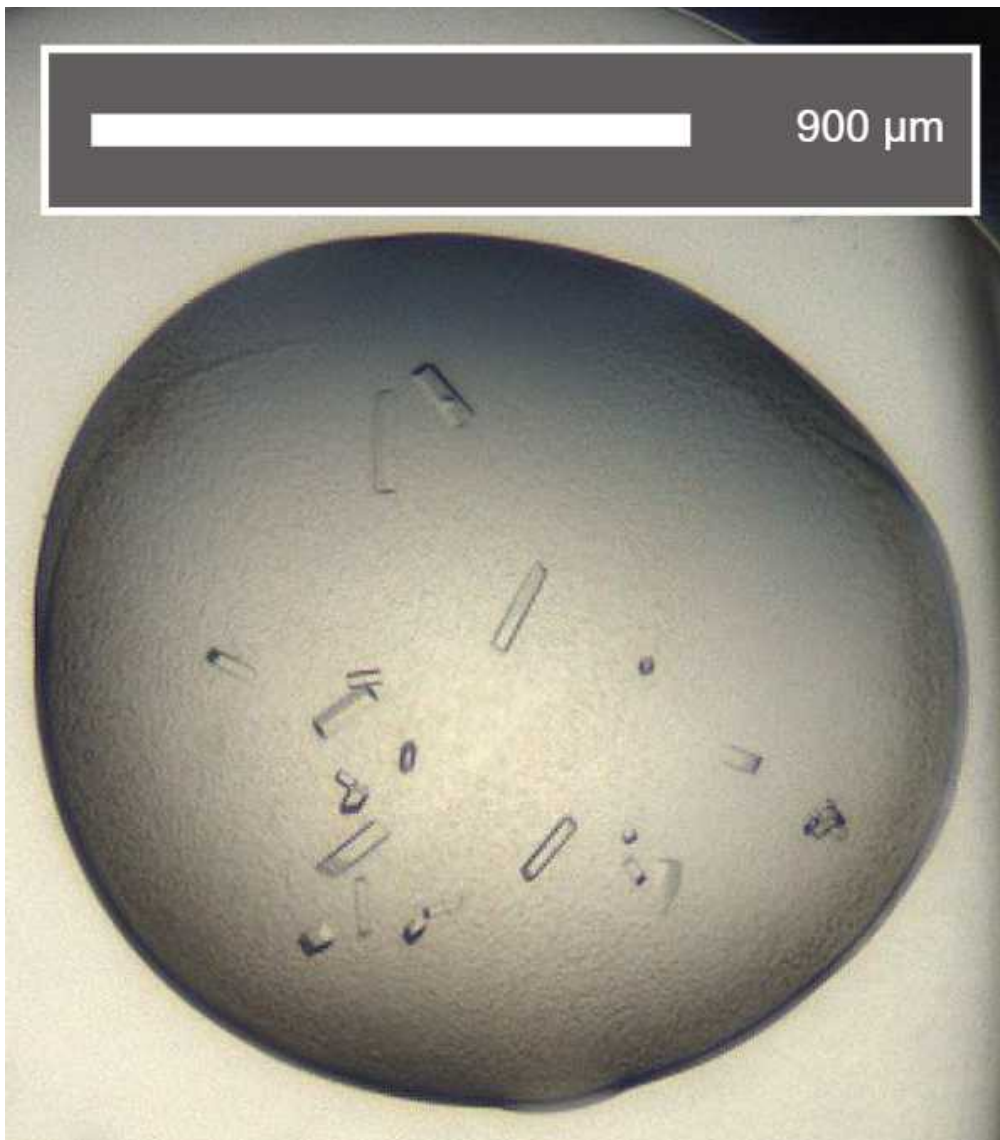
Morphology: typically rectangles.

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 $\text{\AA}$

Space group: C2

Unit cell: 86 $\text{\AA}$, 57 $\text{\AA}$, 32 $\text{\AA}$
90°, 95°, 90°



An example of a drop containing Cocksackievirus 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

Protocol references

Crystallographic Fragment Screen of Cocksackievirus A16 2A Protease identifies new opportunities for the development of broad-spectrum anti-enterovirals, <https://doi.org/10.1101/2024.04.29.591684>