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Crystallization of Enterovirus coxsackievirus A16 2A protease

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ryan Lithgo[1,2], Peter Marples[1,2], Lizbé Koekemoer[3], Daren Fearon[1,2]

[1]Diamond Light Source; [2]Research Complex at Harwell; [3]Centre of Medicines Discovery, University of Oxford

ryan Lithgo: The principle crystallographer on the Coxsackievirus A16 project.;

ASAP Discovery

Lizbé Koekemoer
University of Oxford

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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 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/**

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	PREVIEW
Peter Marples	

1: 1000 dilution Sample seeds

3 **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

4 **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.

5 Dispense 35 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl 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

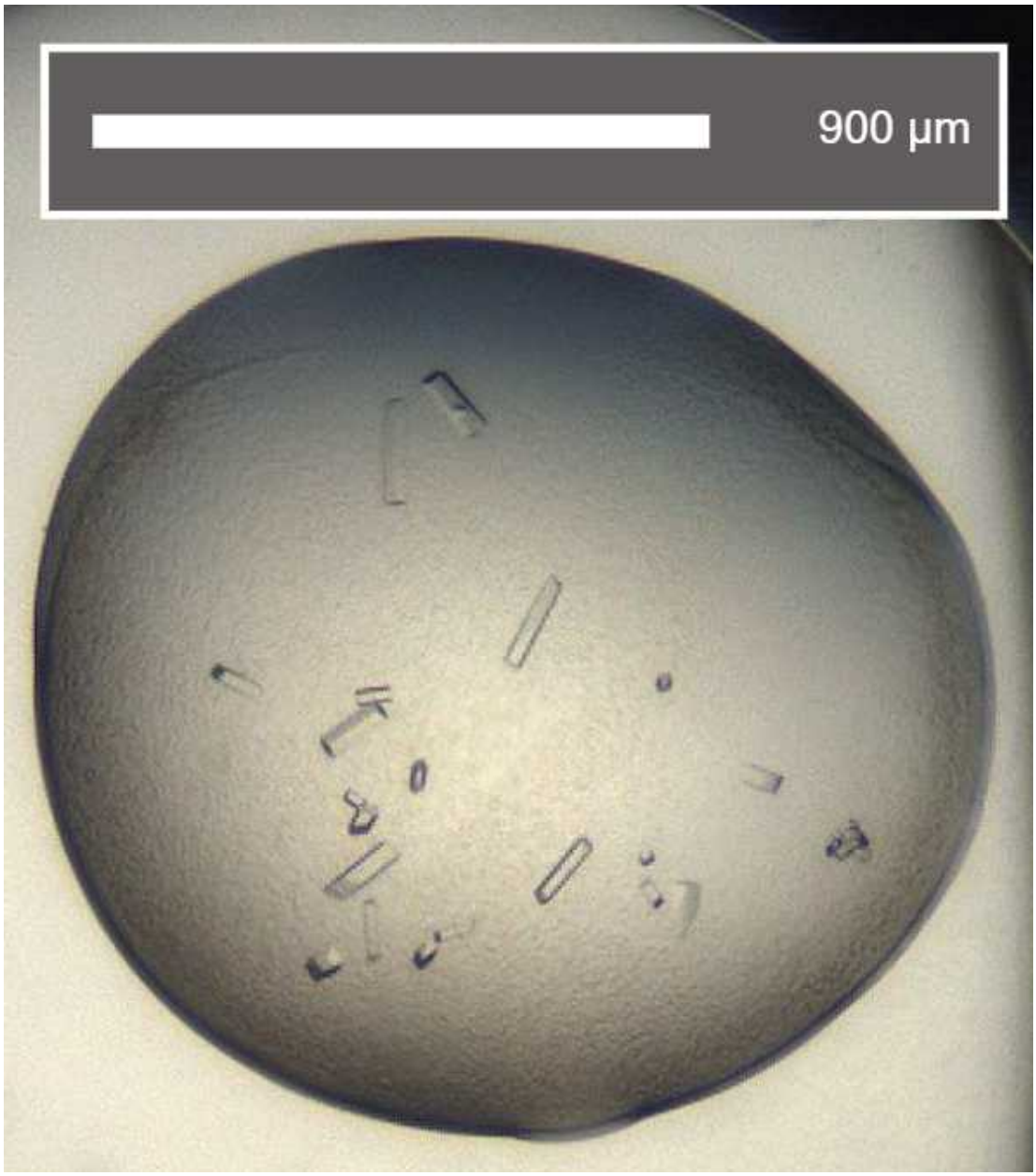
6 Incubate at 20 °C for 24:00:00 h in Formulatrix Rock Imager. 1d

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

7 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

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): 1.21
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

N/A