

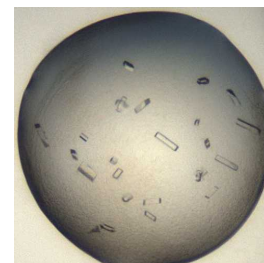
Apr 26, 2024

Version 1

Crystallization of Enterovirus coxsackievirus A16 2A protease V.1

DOI

dx.doi.org/10.17504/protocols.io.3byl49kdzgo5/v1



Ryan Lithgo^{1,2}, Peter Marples^{1,2}, Lizbé Koekemoer³, Daren Fearon^{1,2}

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

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

ASAP Discovery



Lizbé Koekemoer

University of Oxford

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.3byl49kdzgo5/v1>

Protocol Citation: Ryan Lithgo, Peter Marples, Lizbé Koekemoer, Daren Fearon 2024. Crystallization of Enterovirus coxsackievirus A16 2A protease. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.3byl49kdzgo5/v1>



License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: April 26, 2024

Last Modified: April 26, 2024

Protocol Integer ID: 98841

Keywords: crystallisation, XChem, ASAP, AViDD, CMD, Diamond Light Source, i04-1, Coxsackievirus, A16, enterovirus coxsackievirus a16 2a protease picornaviridae coxsackievirus a16, coxsackievirus a16 crystal, 2a protease picornaviridae coxsackievirus a16, enterovirus coxsackievirus a16, 2a protease of the virus, coxsackievirus a16, assembly of capsid protein, final stages of viral replication, capsid protein, virus, antiviral activity, cleavage from the poly protein, viral replication, formation of mature virion, throughput crystallographic fragment screening on the target, crystallographic fragment screening, poly protein, crystallization, 2a protease, protein, protease, target for pandemic preparedness, scale outbreak, host cell

Funders Acknowledgements:

National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAID)

Grant ID: Grant ID: U19AI171399

Disclaimer

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Acknowledgements:

Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot OX11 0QX, UK
Research Complex at Harwell, Harwell Science and Innovation Campus, Didcot OX11 0FA, UK
Oxford Lab Technologies crystal shifter <https://doi.org/10.1107/S2059798320014114>

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/>

Troubleshooting

Safety warnings

 Follow all handling warning for the chemicals used in the crystallisation 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

Peter Marples

Preview

1: 1000 dilution Sample seeds

- 3 **Protein and buffer requirements:**



43.2 μ L



[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 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

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.

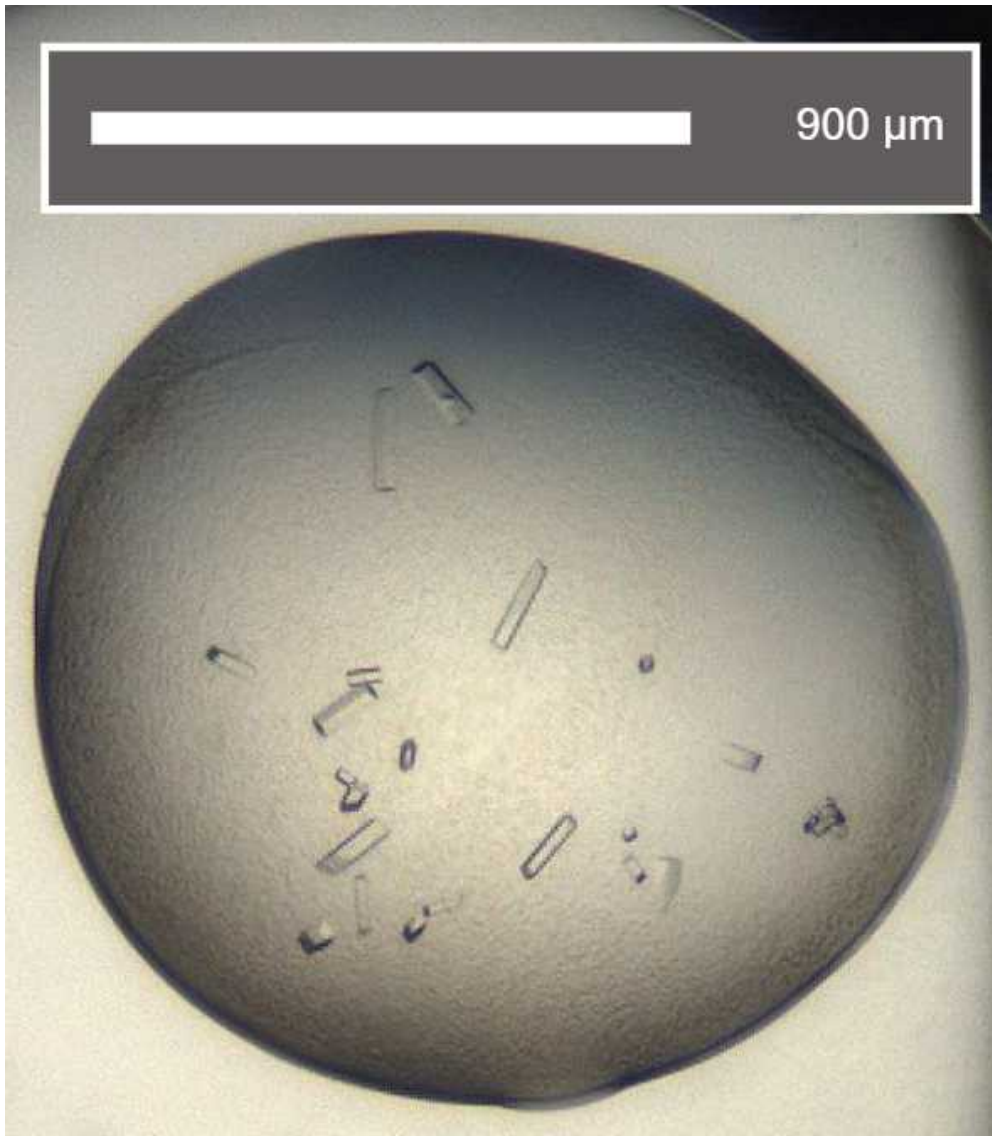
Morphology: typically rectangles.

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 $\text{\AA}$

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

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