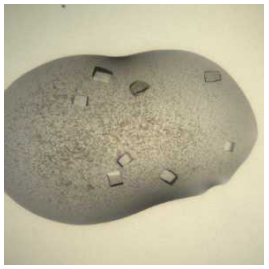
Jul 25, 2025 Version 1

Crystal structure of Enterovirus A71 2A protease mutant C110A containing VP1-2A junction in the active site apo structure (C1 PDB: 9FGO) used for fragment screen and compound soaking V.1



Forked from Crystallization of Zika virus NS2B-NS3 protease (closed conformation in space group P4322 | PDB ID: 8PN6)

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Xiaomin Ni: The principle crystallographer on the 2A protease precursor project.;

ASAP Discovery

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We use this protocol and it's working

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Disclaimer

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Abstract

This protocol describes the crystallization of Enterovirus 71 (EV-71) 2A protease mutant C110A containing the VP1-2A junction in the active site. The crystals form within 12-24 hours using a crystallization screen composed of 1.8M NaCl and 15% ethanol. The crystal structure was determined using X-ray diffraction, resulting in hexagonal prism crystals in space group P61 with unit cell dimensions of 61.5Å, 61.5Å, 78.9Å (90.00°, 90.00°, 120.00°) and an average resolution of 1.5Å. The protein was expressed using the plasmid Enterovirus Coxsackievirus A71 2A protease 228633.

Materials

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

[M] 1 Molarity (M) Ammonium sulfate, Molecular Dimensions, Catalog # MD2-250-35

[M] 1 Molarity (M) Sodium acetate pH 4.8 , Molecular Dimensions, Catalog # 133225

50% w/v PEG 2000, Molecular Dimensions, Catalog # MD2-250-17

Purified protein xxx. 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/204791/**

Protocol materials

Enterovirus Coxsackievirus A71 inactive 2A protease with mutation C110A to preserve VP1-2A junction. **addgene Catalog #**228633

Safety warnings

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

Protein expression and purification

1 The protocol used for protein expression and purification is the following. Using the plasmid

Enterovirus Coxsackievirus A71 inactive 2A protease with mutation C110A to preserve VP1-2A junction. **addgene Catalog #**228633

Equipment needed

2 **Formulatrix Rock Imager**
SPT mosquito
Liquidator 96 5-200 µl pipette
SwissCI 3 lens plate

Protein sequence

3 **ITTLGKFGQQSGAIYVGNFRVVNRHLATHNDWANLVWEDSSRDLLVSSTTAQGCDTIARCN CQTGVYYCNSMRKHYPVSFSKPSLIFVEASEYYPARYQSHLMLAVGHSEPGDAGGILRCQH GVVGIVSTGGNGLVGFADVRDLLWLDDEAMEQQ**

Crystallization experiment

1d

4 **Crystallisation screen composition:**
1.8M NaCl
15% ethanol

Stock solutions used:

[M] 5 Molarity (M) Sodium chloride

[M] 100 % volume Ethanol

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 **Protein and buffer requirements:**
43.2 µL [M] 15 mg/mL Sample
2.88 mL Crystallization screen

6 Dispense 20 µL Crystallisation screen into SwissCI 3 lens plate reservoir wells using a 100 µl multi-channel pipette.
Dispense 100 nL nl [M] 15 mg/mL Sample to each lens using the SPT mosquito.
Dispense 100 nL Crystallisation screen to each lens using the SPT mosquito.

Drop ratio: 1:1
Final drop volume: 200 nl

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

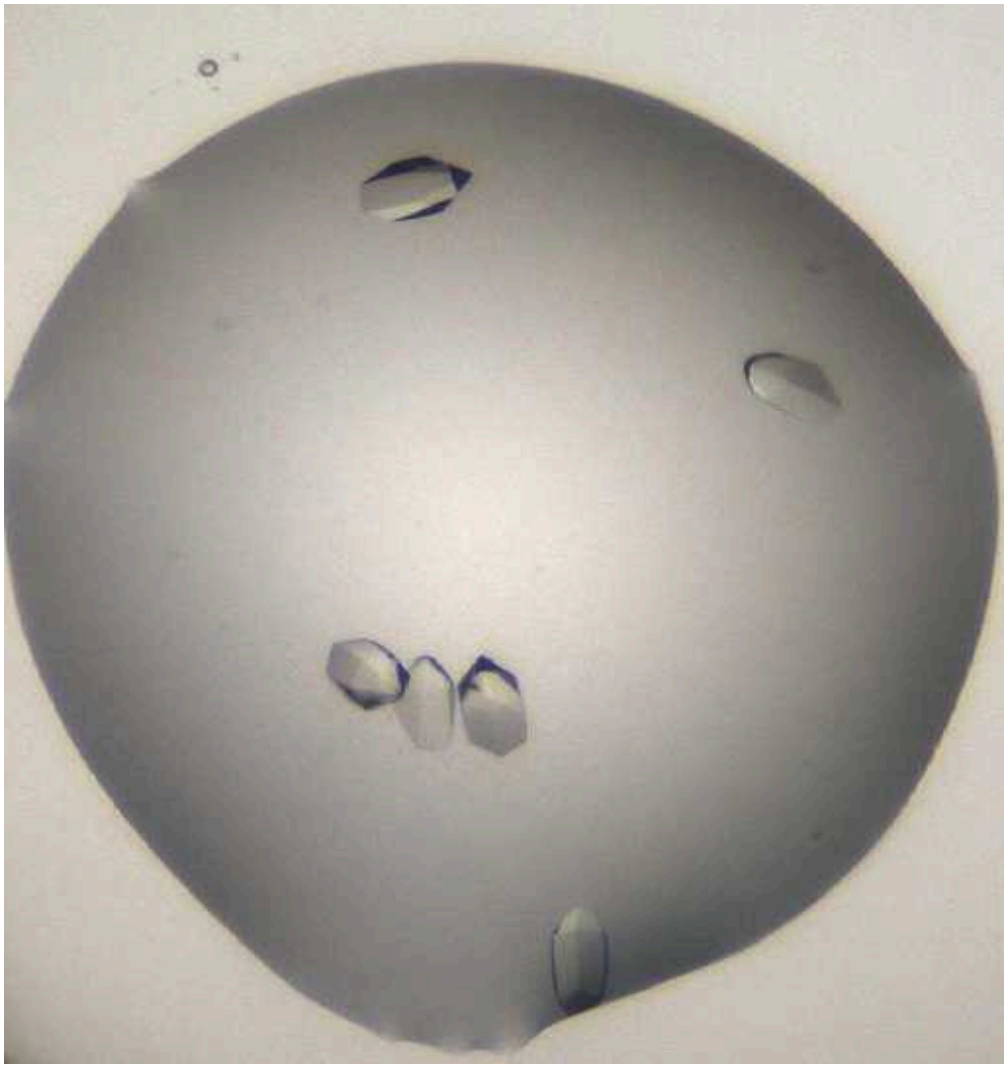
Imaging Schedule: The first images are taken after 12 h and the imaging schedule follows a Fibonacci sequence of days for further collections.

8

Expected result

Crystals typically form within 12 h, within 24 h they have reached their maximum size. Crystals form on their own and have hexagonal prism appearance. (see image below)

Morphology:
Size: ~50 µm in width and ~100 µM in length
Average resolution: 1.5 Å
Space group: P61
Unit cell: 61.5 Å, 61.5 Å, 78.9 Å
90.00°, 90.00°, 120.00°



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): 1.62
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 screen

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

Protocol

NAME

XChem crystallographic fragment screening

CREATED BY

Peter Marples

Preview

Protocol references

N/A