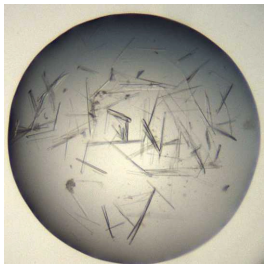
Apr 26, 2024

Crystallization of MERS-CoV Mpro



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blake.h.balcomb: The principle crystallographer on the MERS Mpro project.;

ASAP Discovery

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OPEN ACCESS

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External link: https://asapdiscovery.org/outputs/target-enabling-packages/#ASAP-COV-MPRO

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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

The COVID-19 pandemic has highlighted the need to identify novel therapeutic interventions and strategies for pandemic preparedness. Other than Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), there are several human coronaviruses that are of pandemic concern, these include SARS-CoV and Middle Eastern Respiratory Syndrome (MERS-CoV). MERS-CoV is a zoonotic virus that was first discovered in 2012. The disease has spread rapidly with large outbreaks as recent as 2015 and 2018. Currently there is no therapeutic intervention for MERS-CoV with 35% of reported cases resulting in human death. Like-wise to SARS-CoV-2, MERS-CoV produces a main protease (Mpro) which is essential for viral replication and therefore an attractive target to inhibit the virus.

Materials

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

JCSG+ condition 2-30, Molecular Dimensions, Catalog # MDSR-37-250-2-30

Purified MERS-CoV Mpro protein ([M] 17 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

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:**

17m 40s

Protocol	
NAME	**Diamond XChem Seeding Protocol**
CREATED BY	**Peter Marples**
PREVIEW	

1: 1000 dilution Sample seeds

3 **Protein and buffer requirements:**

32 µL [M] 17 mg/mL Sample
3.36 mL Crystallization screen

14.4 µL Sample seeds, dilution 1:1000

4 **Crystallisation screen composition:**

[M] 0.2 Molarity (M) Sodium malonate dibasic monohydrate

20% w/v PEG 3350

Stock solutions used:

JCSG+ condition 2-30

> **Note**
>
> 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. 10m

Dispense 150 nL [M] 17 mg/mL Sample to each lens using the SPT mosquito.

Dispense 20 nL Seeds to each lens using the SPT mosquito.

Dispense 130 nL Crystallisation screen to each lens using the SPT mosquito.

Drop ratio: 15:13:2 ratio (150 nl Sample : 150 nl reservoir solution: 20 nl seeds)

Final drop volume: 300 nl

6 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.

7 Crystal form after ~24 h.

Expected result

The crystals reach their maximum size after 24 h.

Morphology: typically thin needles or rectangles with pointed ends.
Size: ~100 µm in length and ~2 µm in width, depth of the crystals is ~2 µm
Appearance: glass shard.
Average resolution: 2.2 Å
Space group: $C222_1$
Unit cell: 87 Å, 94 Å, 155 Å
90.00°, 90.00°, 90.00°

An example of a drop containing MERS-CoV Mpro 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): 1.78
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