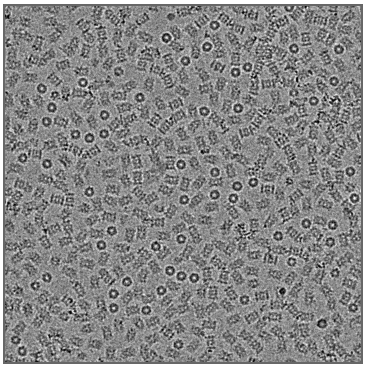

FEB 06, 2024

DOI:
dx.doi.org/10.17504/protocols.io.x54v9px14g3e/v1

Protocol Citation: Frank Adolf 2024. Structural Analysis of 20S CPs and Assembly Intermediates by Electron Cryo-Microscopy. **protocols.io** https://dx.doi.org/10.17504/protocols.io.x54v9px14g3e/v1

MANUSCRIPT CITATION:
https://www.biorxiv.org/content/10.1101/2024.01.27.577538v1

Structural Analysis of 20S CPs and Assembly Intermediates by Electron Cryo-Microscopy

In 1 collection

Frank Adolf[1]

[1]Department of Molecular Machines and Signaling, Max Planck Institute of Biochemistry, 82152 Martinsried, Germany

ASAP Collaborative Research Network

Frank Adolf
Department of Molecular Machines and Signaling, Max Planck I...

ABSTRACT

This protocol details methods for structural determination by transmission electron cryo-microscopy of 20S CPs and assembly intermediates.

GUIDELINES

Please familiarise yourself with the laboratory safety rules and guidelines and follow these while performing the experiment. Please wear appropriate PE while performing the experiment.

MATERIALS

QUANTIFOIL® R 1.2/1.3 Cu 200 - Quantifoil

SAFETY WARNINGS

Liquid nitrogen (LN2) and other cryogens can cause severe damage to the skin and eyes. Always wear personal protective equipment when handling these cryogens.

Protocol status: Working
We use this protocol and it's working

Created: Feb 05, 2024

Last Modified: Feb 06, 2024

PROTOCOL integer ID: 94722

Keywords: ASAPCRN, proteasome, core particle, 20S proteasome, chaperone, molecular machine, multiprotein complex, POMP, PAC1, PAC2, PAC3, PAC4, propeptide, protease

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000282

Plung freezing of 20S CPs amd 20S CP assembly intermediates

1h

1 Prepare Vitrobot and grids for plunging 48s

- Set up Vitrobot as follows: blot force = 3, blot time = 00:00:03 sec, humidity 100%, temperature 4 °C
- Plasma clean Quantifoil R1.2/1.3 Cu 200 grids for 00:00:45 sec , just before plunging

1.1 Apply 3.5 µL of purified 20S CPs at a concentration of [M] 0.5-0.6 mg/mL or purified and concentrated 20S CP assembly intermediates at a concentration of [M] 4.0-5.0 mg/mL with Fos8-cholin at a finale concentration of [M] 0.25 millimolar (mM) on grids, automatically blot and plunge in ethane/propane mix at -180 °C with a Vitrobot Mark IV 1h

1.2 Clipp and store grids in LN2 until screening/data collection

cryo-EM screening and data acquisition

1d

2 Screen cryoEM grids for particle density and ice quality on a Glacios cryo-TEM (Thermo Fisher Scientific) or cryo-TEM of your choise

1d

3 Data collection was carried out either on a Glacios cryo-TEM (Thermo Fisher Scientific) operated at 200 kV equipped with a K2 Summit direct electron detector (DED) camera (Gatan) or Titan Krios G2 cryo-TEM (Thermo Fisher Scientific) operated at 300 kV equipped with a Bio Quantum post-column energy filter (Gatan, 10eV) and K3 direct electron detector (DED) camera (Gatan)

2d

Data collection on both on the Glacios and Titan Krios G2 cryo-TEM and was set up with SerialEM version 4.1 utilizing coma-corrected beam-image shift

CITATION

Mastronarde DN (2005). Automated electron microscope tomography using robust prediction of specimen movements..

LINK

https://doi.org/

Glacios datasets where recorded with one movie per hole in counting mode with a 3x3 or 5x5 multi hole record acquisition scheme at a pixel size of 1.181 Å/pixel with a nominal magnification of 36000x, or at a pixel size of 1.885 Å/pixel with a nominal magnification of 22000x

A total dose of 60 e-/Å2 was fractionated over 40 frames, with a target defocus range of -1.0 µm to -2.6 µm

Krios datasets where recorded with three movie per hole in counting mode with a 5x5 multi hole record acquisition scheme at a pixel size of 0.8512 Å/pixel with a nominal magnification of 105000x

A total dose of 68 e-/Å2 was fractionated over 30 frames, with a target defocus range of -1.0 µm to -2.6 µm

Processing

4 All data processing steps were performed with cryoSPARC version 4.266

2w

CITATION

Punjani A, Rubinstein JL, Fleet DJ, Brubaker MA (2017). cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination..
LINK
https://doi.org/10.1038/nmeth.4169

Raw movies from Glacios datasets were patch motion corrected in cryoSPARC, and raw movies of the Titan Krios K3 dataset were on the fly motion corrected with FOCUS and subsequently imported into cryoSPARC

CITATION

Biyani N, Righetto RD, McLeod R, Caujolle-Bert D, Castano-Diez D, Goldie KN, Stahlberg H (2017). Focus: The interface between data collection and data processing in cryo-EM..
LINK
https://doi.org/10.1016/j.jsb.2017.03.007

All subsequent processing steps were preformed in cryoSPARC, for detaied processing shemes see the Extended Data Figures in https://www.biorxiv.org/content/10.1101/2024.01.27.577538v1.full

Final post-processing was preformed with DeepEMhancer

CITATION

Sanchez-Garcia R, Gomez-Blanco J, Cuervo A, Carazo JM, Sorzano COS, Vargas J (2021). DeepEMhancer: a deep learning solution for cryo-EM volume post-processing..
LINK
https://doi.org/10.1038/s42003-021-02399-1

Model building and refinement

1w

5

AlphaFold2 models of PAC1-4 and POMP along with corresponding chains from a published model of the 20S CP (PDB 5LE5) were manually docked with ChimeraX version 1.5

1w

CITATION

Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Žídek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, Senior AW, Kavukcuoglu K, Kohli P, Hassabis D (2021). Highly accurate protein structure prediction with AlphaFold..
LINK
https://doi.org/10.1038/s41586-021-03819-2

CITATION

Jumper J, Hassabis D (2022). Protein structure predictions to atomic accuracy with AlphaFold..
LINK
https://doi.org/10.1038/s41592-021-01362-6

CITATION

Goddard TD, Huang CC, Meng EC, Pettersen EF, Couch GS, Morris JH, Ferrin TE (2018). UCSF ChimeraX: Meeting modern challenges in visualization and analysis..
LINK
https://doi.org/10.1002/pro.3235

Atomic models build in Coot version 0.9.8.7

CITATION

Emsley P, Lohkamp B, Scott WG, Cowtan K (2010). Features and development of Coot..
LINK
https://doi.org/10.1107/S0907444910007493

Refinment was carried out in Phenix version 1.19.2 and ISOLDE

CITATION

Liebschner D, Afonine PV, Baker ML, Bunkóczi G, Chen VB, Croll TI, Hintze B, Hung LW, Jain S, McCoy AJ, Moriarty NW, Oeffner RD, Poon BK, Prisant MG, Read RJ, Richardson JS, Richardson DC, Sammito MD, Sobolev OV, Stockwell DH, Terwilliger TC, Urzhumtsev AG, Videau LL, Williams CJ, Adams PD (2019). Macromolecular structure determination using X-rays, neutrons and electrons: recent developments in Phenix..
LINK
https://doi.org/10.1107/S2059798319011471

CITATION

Croll TI (2018). ISOLDE: a physically realistic environment for model building into low-resolution electron-density maps..
LINK
https://doi.org/10.1107/S2059798318002425

6