



Report for **Cryo-EM** time at ESRF 1-3 September 2020

Summary:

This continuing project aims to discover the activation mechanism(s) of this protein. Recently, (now published in eLife <https://elifesciences.org/articles/62021> “Structural basis for SARM1 inhibition and activation under energetic stress”) we determined the 3D structure of the SARM1 (sterile α and HEAT/armadillo motif-containing protein) ring octamer in an inhibitory conformation, and found that SARM1 is kept inactive through a ‘substrate inhibition’ mechanism, where physiologic concentration of NAD⁺ stabilizes the tightly packed, inhibited conformation of the protein. In this way, SARM1 activation is directly triggered by a decrease in the concentration of NAD⁺ (a hallmark of several stress conditions), and not necessarily by the introduction of an activating factor. SARM1 gains NADase activity upon the infliction of injury (axotomy), oxidative (mitochondria depolarization; oxidizing agents), metabolic (depletion of NAD⁺), or toxic (chemotherapy drugs) stress conditions. Whether and how all or some of these insults converge to induce SARM1 activation is still not completely understood.

In this session, to learn more about the activation mechanism of SARM1, we continue to investigate how SARM1 small molecule inhibitors that we have identified using high throughput screens affect the SARM1 structure.

Experimental technique(s), required set-up(s), measurement strategy, sample details (quantity...etc)

Regular cryo-TEM single particle collection. Avoid thin ice, and focus on thicker ice regions, where fully-assembled particles can be observed.

Prior to the September 2021 Krios ESRF session: We had a session in November 2019, two more in Feb 2020, one in July 2020, and one in December 2020. Together, we were able to generate a 2.88Å resolution 3D reconstruction of intact, ligand-free SARM1, and a higher-resolution 2.68Å structure of a NAD⁺ complexed SARM1.

September 1-3 2021 ESRF Krios data collection session report

In this session, Dr. Michael Hons, a collaborator of this project, screened through several grids that varied in protein concentration and ice thickness. Data collection was very good.

Parameters: Date 03/09/2021 Proposal MX2378 #of Grids loaded/Screened 8/8 Phase Plate Position #of images/hole 2 #of images collected 7215 Speed 350 #of holes skipped 1 Mag 105k C2 70 Spotsize 5 Dose rate 13.3 No.of frames 40 Exp time 2.2 pixel size 0.84 Total Dose 41.46 Dose/frame 1.036 Obj aperture 100 Grid type Q1.2/1.3 LC Michael FEG Emission 273

Processing report

We have used cryoSPARC v2 for CTF correction and particle picking, iterative 2D classification, and 3D ab-initio reconstructions and refinement. ~50,000 particles were used for 3D reconstruction.

The 3D model resolution is 4Å. This allows us to position all the secondary structure elements.

Publications

Based on the results that we have collected in the previous two Krios sessions, we have now submitted a manuscript to eLife

“A Duplex Structure of SARM1 Octamers Induced by a New Inhibitor”