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Report for Cryo-EM time at ESRF 4-6 December 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 begun 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 December 2020 Krios ESRF session: We had a session in November 2019, two more in Feb 2020, and one in July 2020. Together, we were able to generate a 2.88A resolution 3D reconstruction of intact, ligand-free SARM1, and a higher-resolution 2.68A structure of a NAD+ complexed SARM1.

December 4-6 2020 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 excellent.

Parameters: Date 04/12/2020 Proposal MX2312 #of Grids loaded/Screened 10/10 Phase Plate Position #of images/hole 2 #of images collected 8260 Speed 188 #of holes skipped 656 (one square completely) Mag 165k C2 50 Spotsize 4 Dose rate 8.45 No.of frames 40 Exp time 3.5 pixel size 0.827 Total Dose 43.2428 Dose/frame 1.081 Obj aperture 100 Grid type Q1.2/1.3 LC Michael FEG Emission 207

Processing report
We have used warp for particle picking and cryoSPARC v2 for CTF correction, iterative 2D classification, and 3D ab-initio reconstructions and refinement. ~150,000 particles were used for 3D reconstruction.
The 3D model resolution is 2.9A. This allows us to position all the secondary structure elements and most side chains. Alas, the inhibitor (designated TK190) is not apparent in this reconstruction.

Recent Publications
Based on the results that we have collected in the previous two Krios sessions, we have published a manuscript in eLife
https://elifesciences.org/articles/62021 "Structural basis for SARM1 inhibition and activation under energetic stress"