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Report for Cryo-EM time at ESRF 3-6 February 2020

Summary:
This project aims to determine, for the first time, a 3D structure of the SARM1 (sterile α and HEAT/armadillo motif–containing protein) ring octamer. Using SEC-MALS and negative-stain EM, we have discovered (Sporny et al., 2019) that SARM1, a protein that executes axonal degeneration, forms an octamer ring structure. Using X-ray crystallography, we further found that the SARM1 octamer is arranged around tandem SAM domains. This arrangement was not described before in other SAM proteins, but is reminiscent of the apoptosome and inflammasome - well known ring-like oligomers that like SARM1 - may lead to cell death. **In these CryoEM experiments we aim** to reveal how the catalytic TIR domain is kept auto-inhibited in homeostasis, and what might activate SARM1 under metabolic and oxidative stress. But the most fascinating question is whether there a particular functional relevance for the ring arrangement of SARM1, considering its resemblance to the other degenerative complexes, that is, apoptosome and inflammasome, and in light of the recently discovered interplay between SARM1 and the inflammasome.

Prior to the Feb 2020 Krios ESRF session: We had a three-day Krios session inn November 2019, in which we collected 3700 movies and extracted ~100,000 particles for 3D reconstruction. We applied for the Feb. 2020 Krios session in order to complete the data collection and thus to obtain a final high-resolution reconstruction.

Feb. 3-6 2020 ESRF Krios data collection session report
In this session, grids that were already in CM01 were measured by Dr. Michael Hons, a collaborator of this project, who screened through eight grids that varied in protein concentration and ice thickness. **Due to system failure in CM01 that was resolved only by the last 24 hours of the allocated time,** only a small part of our planed collection was completed.
Date 03-06/02/2020
Proposal MX2247
#of Grids loaded/Screened 2/6
Phase Plate Position 0
#of images/hole 3
#of images collected 4070
Speed 107
#of holes skipped 34
Mag 165k
C2 70
Spotsize 6
Dose rate 4.67
No.of frames 40
Exp time 7
pixel size 0.827
Total Dose 47.79735908
Dose/frame 1.194933977
Obj aperture 100
Grid type QF 1.2/1.3 400 CU
LC Michael
FEG Emission 207 uA

4070 movies were collected and auto-processed for motion correction and dose weighing (DW). I have downloaded the data directly via
rsync -avztuHAXP -e 'ssh -p5022' mx2218@firewall.esrf.fr:
/data/visitor/mx2247/cm01/20200203/RAW_DATA/mx2247_Grid4_sarm1_EPU/Images-Disc1

Processing report

We have used cryoSPARC v2 for CTF correction, particle picking, iterative 2D classification, and 3D ab-initio reconstructions and refinement. Of the 200,000 particles that were used for 3D reconstruction, <100,000 are eventually used for the reconstructing of one homogenous model and were added to the particles previously collected in the Nov. 2019 session.
The 3D model estimated resolution ranges between 2.88-7A. This allows us to position all the domains and most of the secondary structure elements. However, even the best EM maps - the outcome of local refinement protocols **fall (a little) short of revealing some of the most interesting features of the structure**.

Therefore, and because the massive loss of time in the Feb. 2020 session, we immediately request for another 72 hour Krios time, in which data collection will continue on similar grids, to allow the finalization of this exciting project and to bring it to publication.

Sporny, M., Guez-Haddad, J., Lebendiker, M., Ulisse, V., Volf, A., Mim, C., Isupov, M.N., and Opatowsky, Y. (2019). Structural Evidence for an Octameric Ring Arrangement of SARM1. J Mol Biol.