EUROPEAN SYNCHROTRON RADIATION FACILITY
ESRF User Office
CS 40220, F-38043 GRENOBLE Cedex 9, France
Delivery address: 71 avenue des Martyrs, 38000 GRENOBLE, France
Tel: +33 (0)4 7688 2552; fax: +33 (0)4 7688 2020; email: useroff@esrf.fr; web: http://www.esrf.fr

Report for Cryo-EM time at ESRF January 2022

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 January 2022 Krios ESRF session: We had several sessions in CM01. 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.

January 26-28 2022 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. About 10,000 movies were recorded.

Processing report
We have used cryoSPARC v2 for CTF correction and particle picking, iterative 2D classification, and 3D ab-initio reconstructions and refinement. ~150,000 particles were used for 3D reconstruction.
The 3D model resolution is better than 3.5A. 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 published a manuscript
A duplex structure of SARM1 octamers stabilized by a new inhibitor | Khazma T., Golan-Vaishenker Y., Guez-Haddad J., Grossman A., Sain R., Weitman M., Plotnikov A., Zalk R., Yaron A., Hons M., Opatowsky Y. | Cellular and molecular life sciences : CMLS, vol.80, p.16, 2022