	Experiment title: Structural studies on dynein-2 linker remodeling.	**Experiment number**: MX-2261
Beamline: CM01	**Date of experiment**: from: 10/02/2021 to: 12/02/2021	**Date of report**: 22/02/2021
Shifts: 9	**Local contact(s)**: Daouda Traore	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Helgo SCHMIDT, PhD

Department of Integrated Structural Biology
Institute of Genetics and Molecular and Cellular Biology
IGBMC - UMR 7104 - U 1258
1, rue Laurent Fries
BP 10142
67404 ILLKIRCH CEDEX
FRANCE

Report:

We have applied for time on the Titan KRIOS (CM01) as a member of the France BAG (MX2261) coordinated by Laurent TERRADOT. We collected the data on dynein-2 motor domain mutant. This construct is a part of mutation series that we have done on this dynein isoform to demonstrate the contribution of stuctural features to dynein's linker dynamics. So far we have obtained excellent datasets with resolution in the motor domain between 3 to 4 Å. The sample we are working on right now has the H2 insert deleted in the AAA2 domain. Interestingly this particular deletion has casued a strong preferential orientation on the girds, which did not happen to our constructs before. For this reason we have performed screening with several additives on our in-house Glacios microscope, but we haven't found a solid solution to the particle orientation. This is why we asked Daouda (our local contact) if it would be possible to collect the data at 30° tilt and combine this dataset with our previously collected dataset. The tilted images seem to increase the particle distribution (figure 1) to some extent if we compare it to our previous datasets.

The session was scheduled on 10th February and we collected the data remotely by sending the dewar to ESRF 5 days in advance. We prepared 3 quantifoil grids (Cu/Rh 1.2/1.3) with identical concentration and varying ice thickness. During the transport to ESRF grids have been damaged but we were still able to collect data on one of them. Daouda was kind enough to share the atlases of 3 grids and pick the best-looking squares on the grid which did not suffer too much from the transport. The data collection started on Wednesday afternoon and finished on Friday morning.

We have collected around 3000 micrographs, and so far we were able to refine the structure to 6.8Å (Figure 1) without polishing. Exemplary 2D classes shown in figure 2. We will combine the data with the non-tilted dataset soon. We are confident that we will able to reach better resolution. The report will be updated once we perform full data processing..
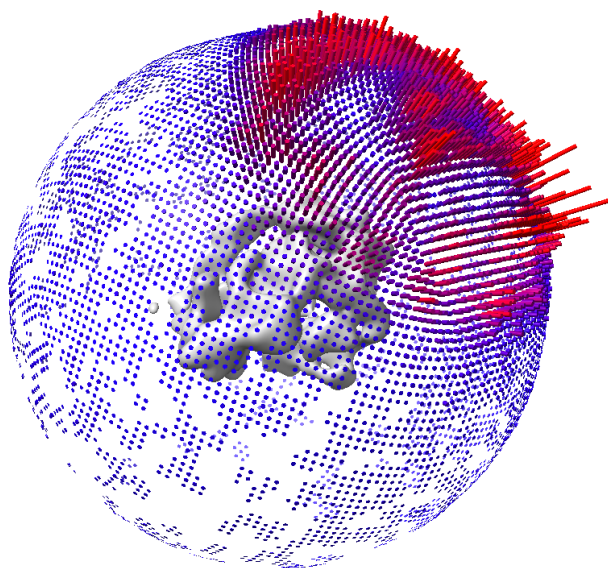
Figure 1. Particle distribution from the tilted images data collection at ESRF.
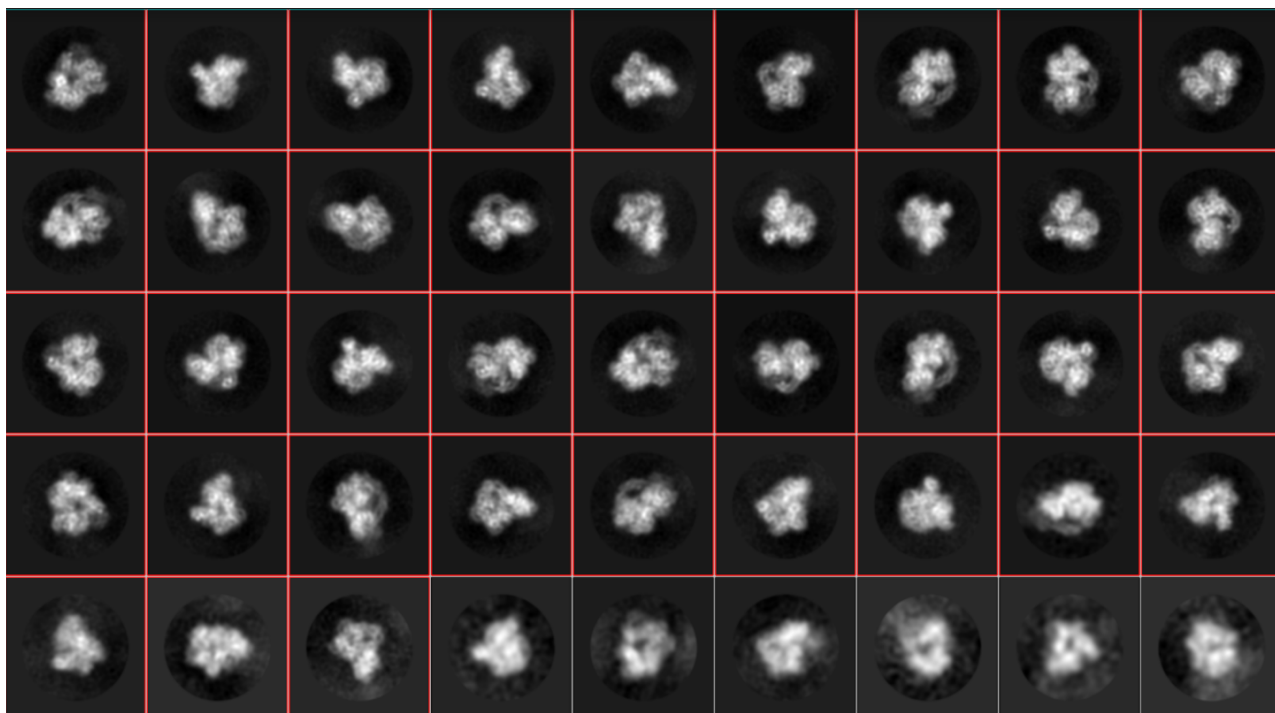

Figure 2. 2D classification outcome after filtering the particles.