	Experiment title: Structural characterization of pseudokinase-containing receptors	**Experiment number**: MX-2507
Beamline: CM01	**Date of experiment**: from: 11/11/22 to: 13/11/22	**Date of report**: 25/08/23
Shifts:	**Local contact(s)**: Eaazhisai Kandiah (email: eaazhisai.kandiah@esrf.fr)	*Received at ESRF:*

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Report:

I applied for time on the titan KRIOS (CM01) through a rolling access (MX-2507). I collected data on a tyrosine kinase receptor-like protein in the aim to decipher its structural mechanisms of activation and regulation. In our lab, we prepared grids of the intracellular part of the receptor and obtained promising 2D classes and 3D reconstruction at 6Å resolution with our in-house 200kV Artica microscope. Following our application for a rolling access to the beamline CM01, we obtained 6 eigth-hour time shifts (two days) to perform an extensive data collection on our best grids with the Titan Krios at the ESRF.

This session was scheduled on November 11th, and we collected the data remotely by sending the dewar to ESRF 4 days in advance. We prepared one grid (Au R1.2/1.3 300 mesh) with our best sample with homogenous particle distribution and varying ice thickness. Due to an issue on the ISPyB website, I was not able to create the shipment and the barcode online. Following the advice from the ESRF user office, I wrote a letter with all the required information, which was sent along with the dewar. The dewar was then correctly handled upon arrival at the ESRF.
After a brief discussion on November 11th with my local contact Eaazhisai Kandiah about the grid quality and the strategy to collect the best cryo-EM dataset, the data collection started on November 11th and finished on November 13th as scheduled.

We collected around 20,000 micrographs, and so far I was able to refine the structure to 3.7 Å resolution (Figure 1). Unfortunately, the 3D reconstruction is so far limited by the preferential orientations of the particles selected, as observed in the particle orientation plot (Figure 1c) and the 2D classes (Fig 2). We believe that this issue is due to difficult particle picking. Indeed, the particles are very small (~110 Å) and they are very crowed in the micrograph, which makes particles picking very difficult. Additionally, it is hard to identify in the 2D classes different orientations other than the "butterfly shape" front view (Fig 2). We are now trying several new particles picking strategy to obtain new particles corresponding to other orientations (i.e., top and bottom view). We are confident that we will be able to reach better resolution and to obtain a high-quality 3D reconstruction. The report will be updated once we perform full data processing.

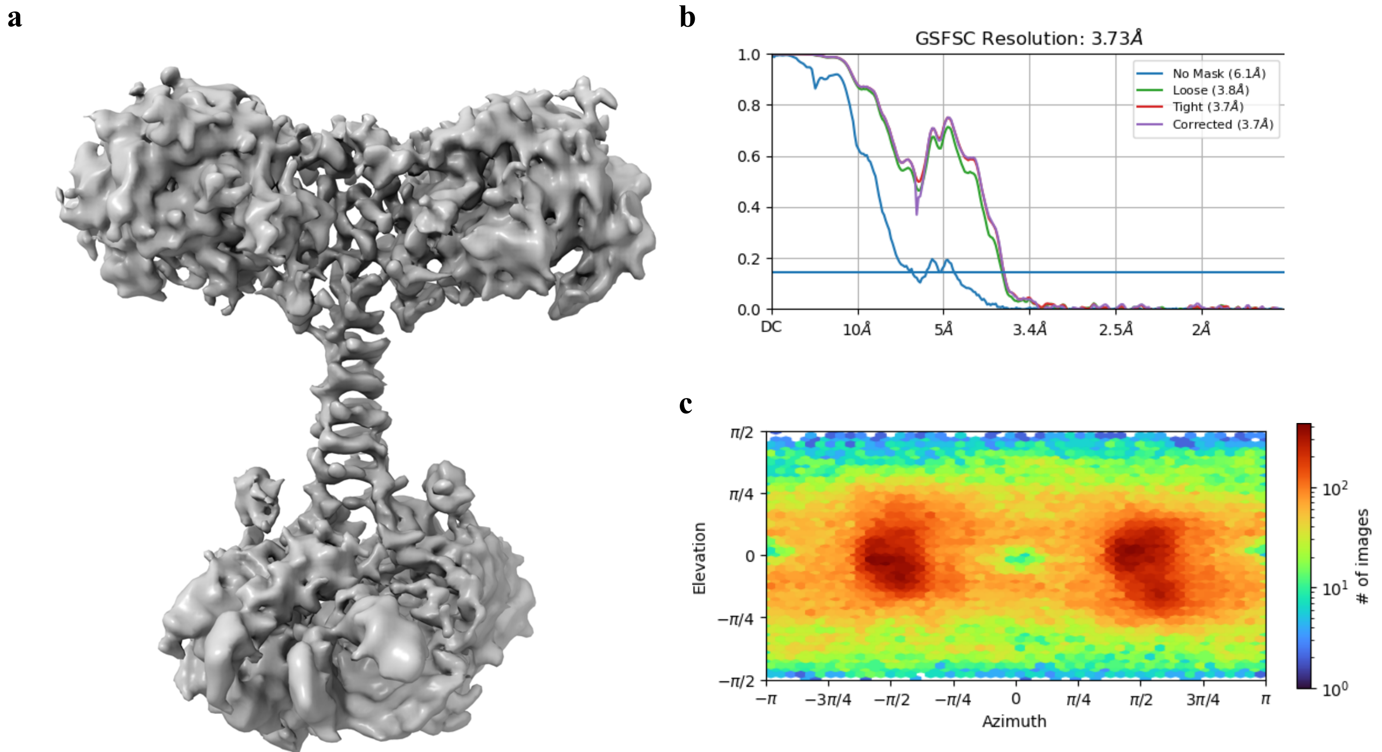

Figure 1: a. Cryo-EM map of the receptor protein **b.** Fourier shell correlation (FSC) plots between half-maps, mask used for determination of the average resolution at FSC 0.143. **c.** Distribution of the particle orientations within the dataset used for 3D reconstruction.

Figure 2: Representative 2D class averages from the final subset of particles used in the reconstruction presented Fig 1a.