ESRF	**Experiment title:** Structural analysis of atypical tripartite toxin-antitoxin-chaperone (TAC) system of M*ycobacterium tuberculosis*.	**Experiment number**: MX2261
Beamline: CM01	**Date of experiment**: from: 10/05/2021 (9AM) to: 12/05/2021 (9AM)	**Date of report**: 02/03/2022
Shifts:	**Local contact(s)**: Daouda Traoure	*Received at ESRF:*

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Report:

As a collaborator of Lionel Mourey (member of the BAG France CryoEM, proposal MX2261, coordinated by Laurent Terradot), we obtained shifts on the Titan KRIOS CM01 between May 10th and 12th 2021, to collect data on atypical tripartite toxin/antitoxin/chaperone (TAC) system of M*ycobacterium tuberculosis.*

In this project, we focus on the atypical three-component toxin-antitoxin system of *Mycobacterium tuberculosis* (Mtb), named TAC (Toxin-Antitoxin-Chaperone). TAC is composed of a highly efficient toxin belonging to the RelE/ParE toxin superfamily, an HTH-Xre antitoxin and a dedicated SecB-like chaperone. In TAC, the role of toxin is played by HigB, which inhibition relies on the stabilization of the of the HigA antitoxine by the SecB chaperone. The SecB-HigA association is essential to prevent the aggregation and the degradation of the antitoxin itself. The objective of this data collection is to resolve the structure of SecB-HigA-HigB complex.

Our local contact was Daouda Traoure. Several grids (Quantifoil R1.2/1.3) were prepared on our local cryo-EM platform (Rennes). Two grids were checked locally to verify the good concentration of the sample and the quality of the ice. The rest of the grids were sent to the ESRF (Fedex). Daouda Traoure clipped 6 grids and loaded them onto the microscope. In remote, we selected together the best grid and the best squares for the acquisition. Three images were collected per selected foilhole, with a pixel size of 0.827 Å and 0.98e^-/Å^2/frame. The collection went smoothly and ended up with 8688 images. The dataset is of very good quality and we thank Daouda Traoure for the excellent processing of our samples, for his expertise and advices.

Collected images were transferred on our local GPU server using rsync. Particles were semi-automatically selected in Cryosparc and subjected to two rounds of 2D-classification in order to remove defective particles. This resulted in the selection of 1,501,287 particles that were transferred into Relion 4.0. Following motion correction (Motion Corr2) and CTF correction (CTFFind 4), 219 movies of poor quality were discarded. An

initial 3-D auto-refinement produced a reconstruction at a resolution of 5.7 Å (without post-processing). To improve the homogeneity, the datasets was then sorted into 10 subsets using the 3D-classification function. This results in 551,718 particles which clearly contain the SecB chaperon. This was followed by a second round of 3D auto-refinement using the same parameters, resulting in reconstructions with resolutions of 5 Å. After two consecutive rounds of 3D-classification, 118,426 particles were finally retained, resulting in a 3.8 Å map, after post-processing.

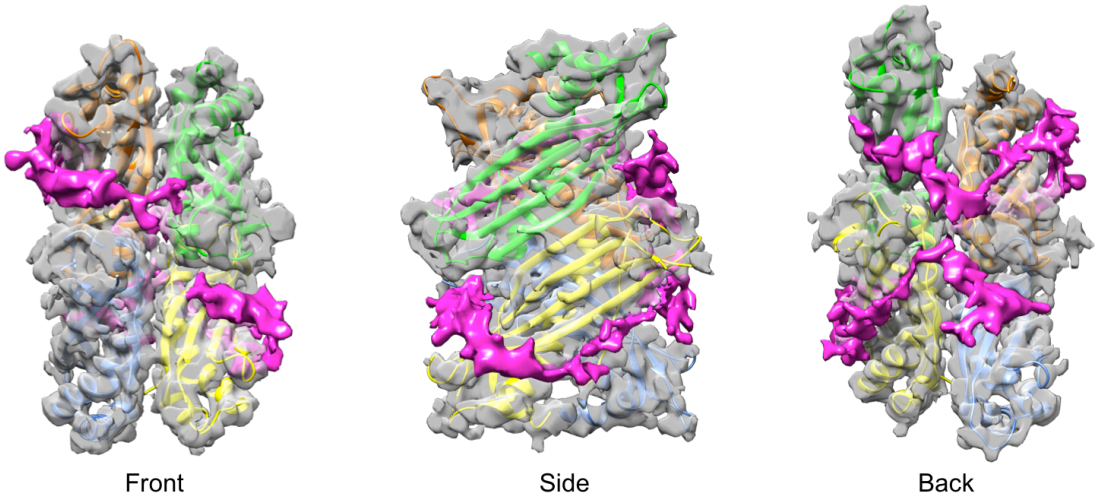

Figure 1 : Comparison between our map and the SecB-like chaperone in complex with a ChAD peptide (PDB: 5MTW). The 4 SecB-like chaperon are colored in blue, orange, yellow and green respectively. The portion of the cryo-em map corresponding to the SecB-like chaperon is transparent while the remaining density, corresponding to the C-terminal extremities of Mtb-HigA1 toxin, are colored in red

Our map contains 4 SecB-like chaperon and two long peptides corresponding to the C-terminal extremities of 2 HigA toxin. Our structure is in good agreement with the crystal structure of the Mycobacterium tuberculosis Rv1957 SecB-like chaperone in complex with a ChAD peptide (PDB: 5MTW), but the native peptide is much longer and is wrap around the SecB tetramer, in agreement with the previously published HDX-MS data (Guillet et al., Nat. Commun., 2019). The analysis of the data is still in progress and we are now re-extracting the particles with a larger box to try to improve the CTF correction and the final resolution. Furthermore, although the reconstruction refined to high resolution, we were not able to identify additional density corresponding to either HigB toxin nor the globular domain of HigA. This could suggest that the toxin was released from the complex during the purification and enticed us to modified our purification protocol in order to obtain more stable complexes.