	Experiment title: **Structure of bacterial MexAB-OprM multidrug efflux pump**	**Experiment number**:
Beamline:	**Date of experiment**: from:03/04/18 to: 06/04/18	**Date of report**: 29/06/18
Shifts:	**Local contact(s)**: Gregory Effantin	*Received at ESRF:*

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Report:

We have applied for 9 shifts on the Titan KRIOS to collect data on the MexAB-OprM tripartite multidrug efflux complex in February 2018 and collect data in April 2018 (From 3 to 6). Our local contact was Gregory Effantin.

We brought several grids, pre-screened on our Arctica microscope. After checking all grids, we select one on which could select several squares suitable for automatic collection (no crack, good ice, good particle concentration). We started the collection at the end of the first day and Gregory continues the data collection for us the next two days. The collection went well and ended up with 3360 images. I would like to emphasise that Gregory was very professional all along the experiment and provided us the good advices and its excellent expertise to get the quality and size of the dataset.

For the image processing, about 300 000 particles automatically selected from 3068 movies. Relion was used for all the data processing. We have obtained a consensus reconstruction at about 3.5 Å resolution with ~ 90 000 particles (Figure 1).. We could fit X-ray models of the three components and can see numerous details at the nanodisc interfaces. We are now in the phase of refining the 3D models.

Among the set of extracted particles, there were some particles that were not the tripartite complex but could potentially be the MexAB bipartite complex. Despite efforts to process those particles, we

did not succeed to have homogenous classes. We are working on preparing the bipartite complex MexAB for getting a complete view of the assembly process. When the sample will be ready, I would like to collect data on this bipartite complex and hence I would like to apply for more time on the Titan KRIOS at ESRF.
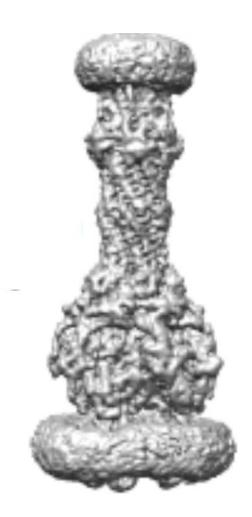

Figure 1 3D reconstrucution of tripartite complexes. We clearly see the three components OprM, MexA , MexB. The nanodisc part is less defined.

We are considering to publish these data . Until then could you please keep the results confidential.