ESRF	**Experiment title: Structural studies on complexes between RNA polymerase and 70S ribosomes**	**Experiment number**: MX-2261
Beamline: CM01	**Date of experiment**: from: 05/10/2020 to: 07/10/2020	**Date of report**: 02/02/2021
Shifts: 9	**Local contact(s)**: Eaazhisai KANDIAH	*Received at ESRF:*

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Report:

We have applied for time on the Titan KRIOS (CM01) as a member of the France BAG (MX2261) coordinated by Laurent TERRADOT. We collect data on functional complexes of a transcribing RNA polymerase (RNAP) elongation complex coupled to a translating 70S ribosome. This assembly is called an expressome. Previous datasets collected at the ESRF were a major contribution to our first paper, which was published in 2020 (Webster et al., *Science* 20202). One question that we now would like to address are alternative RNAP binding sites on the 70S ribosome. We identified these sites in negative stain data and biochemically verified their stability using sucrose gradient centrifugation. We were kindly offered extra time on CM01 and were scheduled for October 2020 (05/10 – 07/10). Our local contact was Eaazhisai KANDIAH and this was a remote session (our samples were shipped prior to the experiment).
We sent 6 Quantifoil R 2/2 grids with identical sample concentrations. Our local contact screened the first 4 grids for us and we had a zoom meeting to identify the best grid for data collection at around 11am the first day. She also kindly sent us images of the Atlases as well as some particle images to inspect so we could make an informed decision. We were able to quickly identify the best grid and our local contact then setup data collection and we were able to follow progress through the webinterface (Extended IsPyB).

As usual, the dataset was superb (Figure 1). We had almost 8,500 images. Individual 70S ribosomes and RNAP particles could be identified. However, even though the reconstruction refined to high resolution, we were not able to identify additional density corresponding to stably bound RNAP on the ribosome. From our negative stain data we know where RNAP should approximately be bound (please keep in mind, this is a different binding site from the one we reported in Webster et al., *Science* 2020), but so far we were not sussesful in idenitifying a (sub-)set of particles with bound RNAP. We will update this report if we make progress.

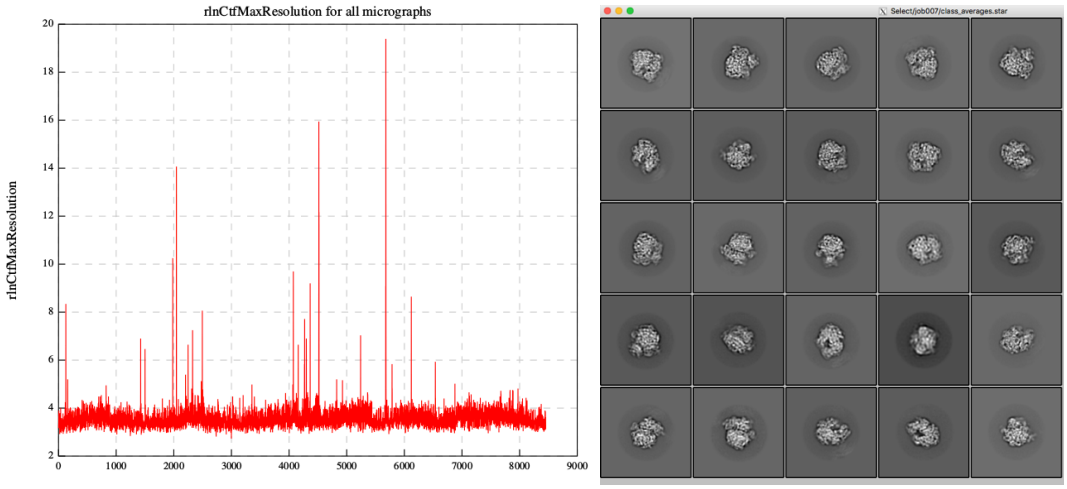

Figure 1.: Resolution estimates after initial data processing suggested the majority of images have information to between 3Å and 4Å. Initial 2D classes looked promising but we did not see extra density corresponding to RNAP bound to the ribosome. We are still trying to