	Experiment title: Structural studies on RNA polymerase ribosome complexes	**Experiment number**: MX-2441
Beamline: CM01	**Date of experiment**: from: 20/02/2023 to:22/02/2023	**Date of report**: 22/05/2023
Shifts:	**Local contact(s)**: Gregory EFFANTIN	*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 collected data on a supramolecular compelx between bacterial RNA polymerase (RNAP) and the small ribosomal subunit (30S). In prokaryotes, transcription and translation are coupled processes. The goal is to understand how transcription can stimulate protein synthesis and thus to understand how RNAP recruits the ribosome to the nascent mRNA.

This was a tricky sample because we have collected multiple datasets before and knew that the target complex is likely rare. However, some recent biochemical resutls gave us confidence and we felt we found a way to enrich the target. We opted for a remote data collection because of the ongoing train strikes (a recently scheduled session on CM01 had to be cancelled because of last minute strikes that did not allow us to get to ESRF in time).

The session was scheduled for February 20th and we collected a very large dataset of more than 25000 movies from two different grids. We think it is an extraordinary service to allow collection from two different grids because it ensures that the best possible squares can be chosen. Our local contact, Gregory EFFANTIN, was in

contact with us before and during the session and this allowed us to pick the ideal squares and holes for efficient collection.

We have now spent the last 3 months analysing and processing the data and it turned out to be very interesting. The large dataset allows us to classify the sample into many different functional states and we can also see RNAP tethered to the ribosome through the nascent mRNA. The 30S easly refines to high resolution (below 3Å) and we believe we can push it much higher. Much more important, we were able to identify classes that had additional density – after focused refinement approaches, we could confirm this to be RNAP in two different positions (refined to 3.8Å and ~7Å, see red and green density in Fig.1). Furthermore, the 30S can also be classified into a number of different fucntional states that were unexpected. We are very excited about these results and it is clear that a superb dataset could be collected thanks to the support from ESRF!

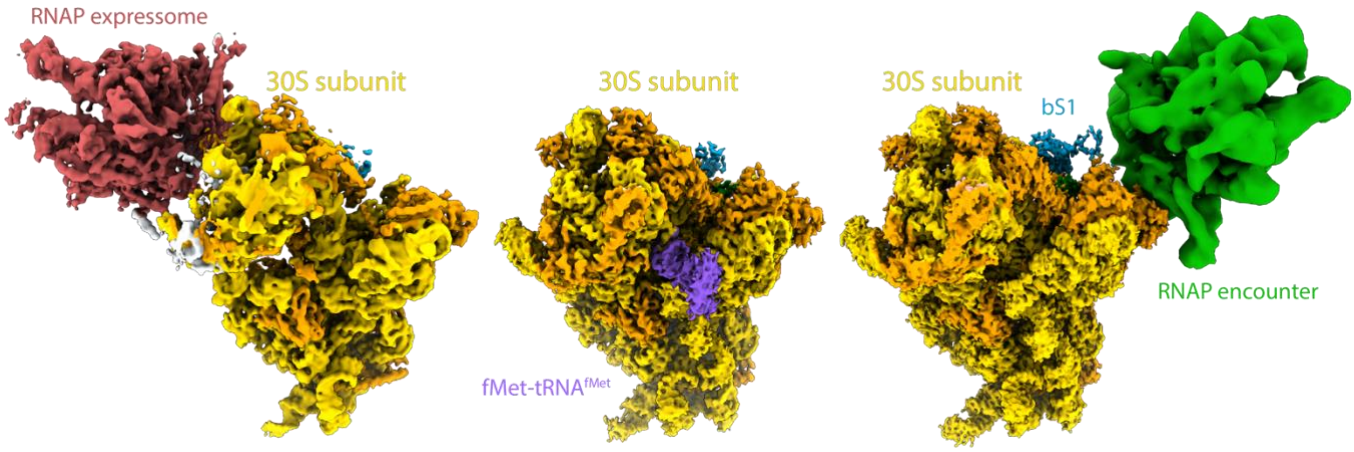

Figure 1: Preliminary results from classifying a 30S-RNAP complex into 3 main classes. RNAP is tethered to the 30S through the shared mRNA. It can either bind in the so-called expressome position (left, RNAP in red, 30S in gold and orange), or to the 30S platform at the mRNA exit channel (right, RNAP in green). A subset of 30S subunits has initiator tRNA bound (center).