**SUPPLEMENTARY INFORMATION**

We previously submitted a proposal in October 2019 where we proposed three separate projects. We were encouraged to submit a proposal for each project which we have now done for the January 5th, 2020 deadline. For this application, we have submitted this new project and including more information on how samples were prepared for EM analysis, including expected structural details that will be derived from high-resolution structures. We have updated the time requested based reviewers' suggestions for ribosome data collections.

**Mechanism of ribosomal frameshifting.**

*Background.* Ribosomes undergo mRNA frameshifting in response to different stresses or as a gene expression mechanism[1]. Typically, complex RNA molecules such as tRNAs and mRNAs influence the ribosomes to shift into a new mRNA frame, resulting in the expression of a protein with a different amino acid sequence. We previously solved snapshots of the ribosomes undergoing frameshifting in response to frameshift-prone tRNAs[2-6]. We propose here to capture two structures of the ribosome with modification-deficient tRNAs known to cause frameshifting[7] and bound to an elongation factor at two defined states during the elongation cycle.

*Preliminary data.* To assemble this ribosomal complex, we followed our previously published protocols[4]. We also biochemically verified that ribosomes complexes contain all ligands added. Three µl of ribosome complexes containing mRNA and P-site $tRNA^{fMet}$ were placed on glow-discharged grids (Quantifoil 1.2/1.3 300 mesh Cu) and blotted for 3 seconds using a Gatan CP3 at >90% humidity. A 1,682 micrograph dataset was collected on an FEI Talos Arctica transmission electron microscope operating at 200 keV with BioQuantum/Gatan K2 direct electron detector. Micrographs were collected using a defocus range of -0.5 to -3.5 µm and a dose of 54.54 $e^-$ per pixel and were acquired as 40-frame movies with 15s exposure time. Pre-processing with MotionCorr2 and Gctf using Relion 3.0 was performed, then semi-autonomous particle picking was used to select ribosomes and incorrectly selected particles discarded after reference-free two-dimensional classification. An initial model was generated from the dataset and used to perform further 3D-classifications to identify particles containing P-site tRNA. Iterative rounds of classification and refinement were performed, followed with CTF Refinement and Bayesian polishing. A final reconstruction of the 70S complex was obtained at ~3.9Å from 56,107 particles and shows all ligands added to the ribosome are bound.

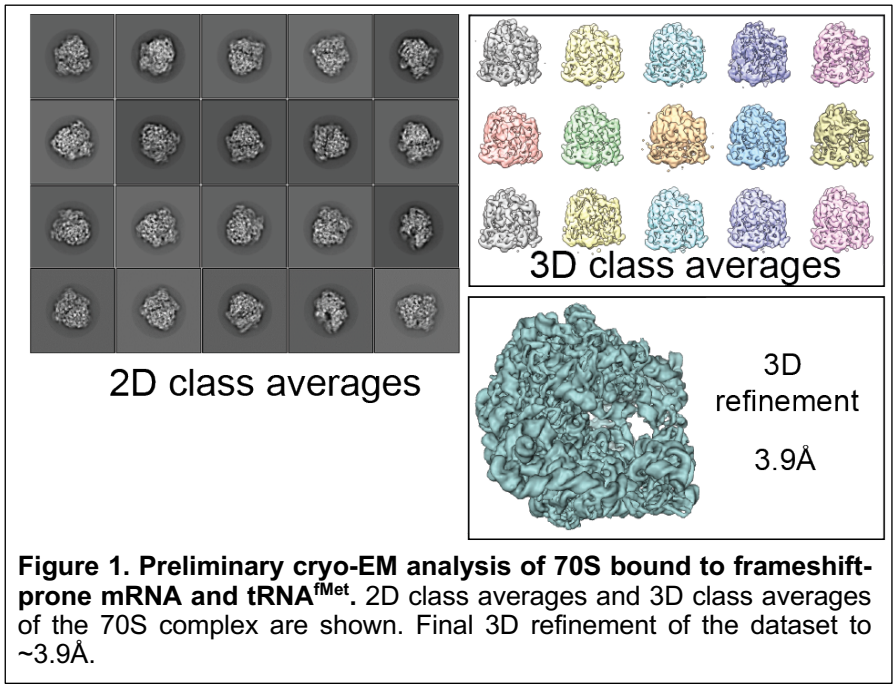


**Figure 1. Preliminary cryo-EM analysis of 70S bound to frameshift-prone mRNA and $tRNA^{fMet}$.** 2D class averages and 3D class averages of the 70S complex are shown. Final 3D refinement of the dataset to ~3.9Å.

*Proposed studies and expected results.* We are limited by our current resolution and require higher resolution to confidently identify the molecular details of these interactions. Our goal is to identify how the ribosome undergoes structural changes of the different tRNA binding sites that might explain how it senses changes that occur between the mRNA-tRNA pair. We also expect to observe large changes of regions of the ribosome known to be dysregulated during frameshifting[4,8,9]. **We request one day for two datasets.**

**References**

[1]Korniy *et al. FEBS letters* **593**, 1468-1482, doi:10.1002/1873-3468.13478 (2019).[2]Fagan *et al.RNA* **20**, 1944-1954, doi:10.1261/rna.046953.114 (2014).[3]Maehigashi *et al.Proc Natl Acad Sci U S A* **111**, 12740-12745, doi:10.1073/pnas.1409436111 (2014).[4]Hong *et al.* Mechanism of tRNA-mediated +1 ribosomal frameshifting. *Proc Natl Acad Sci U S A* **115**, 11226-11231, doi:10.1073/pnas.1809319115 (2018).[5]Zhang *et al. Structure* **26**, 437-445 e433, doi:10.1016/j.str.2018.01.013 (2018).[6]Nguyen *et al. J Biol Chem* **294**, 5281-5291, doi:10.1074/jbc.RA119.007410 (2019).[7]Gamper *et al.Nature communications* **6**, 7226, doi:10.1038/ncomms8226 (2015).[8]Zhou *et al. Proc Natl Acad Sci U S A* **116**, 7813-7818, doi:10.1073/pnas.1901310116 (2019). [9]Chen *et al. Nature*, doi:10.1038/nature13428 (2014).