SUPPLEMENTARY INFORMATION

Preliminary data for each project described in the Abstract are outlined below:

1-RNA modification and antibiotic resistance. With the Conn Lab (see Biosketch), we previously determined the X-ray crystal structure of the pathogen-associated NpmA methyltransferase (MT) and Kmr MT bound to the 30S subunit (PMCID PMC4035980 and West *et al.*, in prep). These studies demonstrated that these functionally equivalent enzymes recognize and modify their the 30S target site in different ways. We are now focused on a more clinically-relevant MT (RmtB) that modifies the 30S at the same surface but a different nucleotide (**Fig. 1A**). Strikingly, to access its target nucleotide, this enzyme appears to disrupt features of the ribosome as assessed by the large movement of the head domain (**Fig. 1B**). In addition, we have preliminary cryoEM structures for another MT (TlyA) that modifies both subunits and that is <u>*required*</u> for tuberactinomycin antibiotic (*e.g.* capreomycin) binding in *M. tuberculosis* (**Fig. 1C,D**).

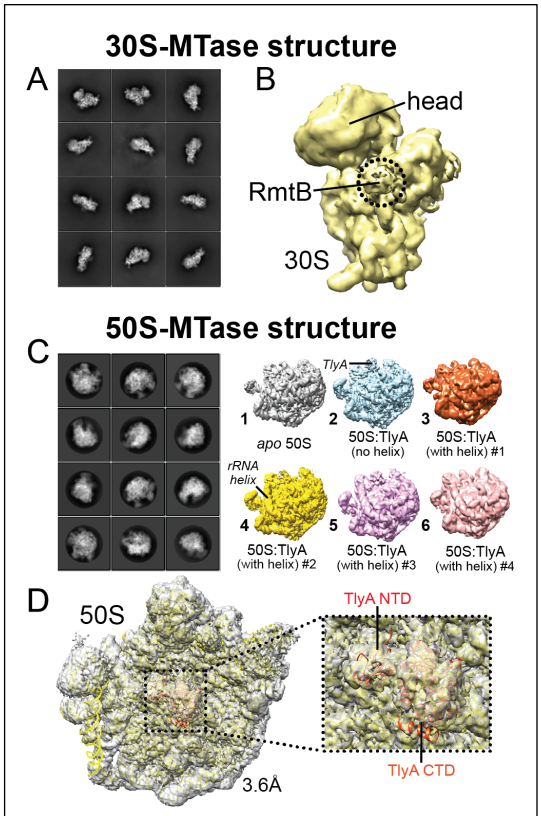

Fig. 1. Preliminary cryo-EM analysis of ribosome-MT complexes for Project 1. A. 2D classifications of 30S-MT. **B.** 3D reconstruction for one class with the extra density in the expected location of the MT (dashed circle; ~4.6Å overall resoltuion). **C.** Sample (12 of ~20) 2D classes of 50S-MT. 3D classification identifying *apo* 50S and multiple MT-bound particle classes. **D.** 50S-MT maps (class 2) with fit *M. smegmatis* 50S structure (PDB 5O60). The MT (TlyA) is manually docked.

2-* Francisella tularensis *70S ribosome rescue by a novel stalling factor. We have collected data on the novel stalling factor ArfT bound to a non-stop mRNA on the *F. tularensis* ribosome with its cognate release factor (**Fig. 2**). Since this stalling factor interacts with both release factors in bacteria (unlike the *E. coli* ArfA), defining the molecular details of its action will provide important insights into potential mechanisms to inhibit *Francisella* (a bioterrorism agent). However, we are limited by resolution and need to collect higher resolution to identify the molecular details of these interactions.

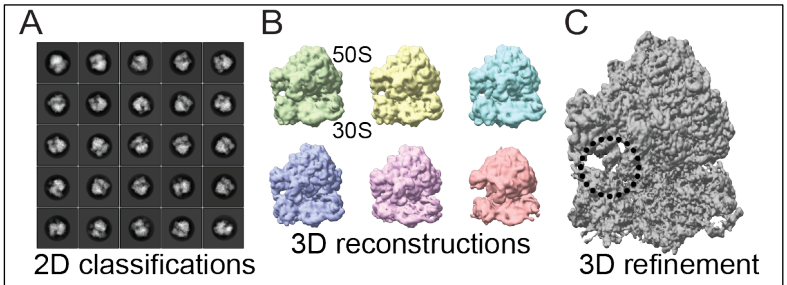

Fig. 2. Preliminary cryo-EM analysis of 70S bound to a novel stalling factor for Project 2. A. 2D classifications. **B.** 3D reconstructions of the 70S at ~4.0Å **C.** 3D refinement of one 70S class to 3.8Å with factor in dashed circle.

3-Changing gene expression in CD8+ T cells by altering ribosome composition. The Ahmed lab at Emory has determined that changing gene expression in CD8+ effector T cells after viral infection results in changes in ribosomal composition (PMID 28714979). In collaboration, we have purified ribosomes from mouse spleen CD8+ T cells at four defined stages after infection where it is known that the composition of the ribosome changes. We collected negative stain images which indicate the ribosomes are homogeneous (**Fig. 3**). Because we know of some important factors that are bound as assessed by mass spectroscopy, we need the highest resolution possible to identify and build these exciting cofactors.

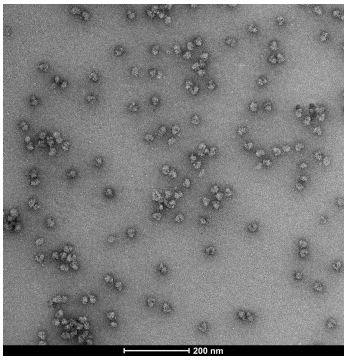

Fig. 3. Preliminary negative images of the mouse ribosomes for Project 3.