Sub-Project 1: Bacterial antibiotic resistance and novel ribosome-targeting antimicrobials. For this sub-project, most materials have been cloned, purified, assembled into functional complexes, and biochemically tested. For example, a new *E. coli* strain was engineered to remove r-protein uL11 to allow the 23S rRNA thiostrepton-resistance methyltransferase TsnR to bind at this site. Preliminary cryo screening data for *Mtb* 30S-TlyA and for MBX-4132 bound to *M. tuberculosis* nonstop 70S ribosomes have been performed (**Fig. 1**). Additional complexes will be built with *Mtb trans*-translation factors (which recognize nonstop ribosomes) and supported by biochemistry. In collaboration with the Keiler lab, *Mtb* persister cells were grown and ribosomes from these cells will be purified. To solve a structure of KKL-1001 bound to the L10/L7/L12 ribosomal stalk, since this region of the ribosome is flexible, our approach is to subclone the L10/L7/L12 operon for expression. This is a higher oligomeric state of ~70-110 kDa (PMID:15989950 of the stalk from another organism).

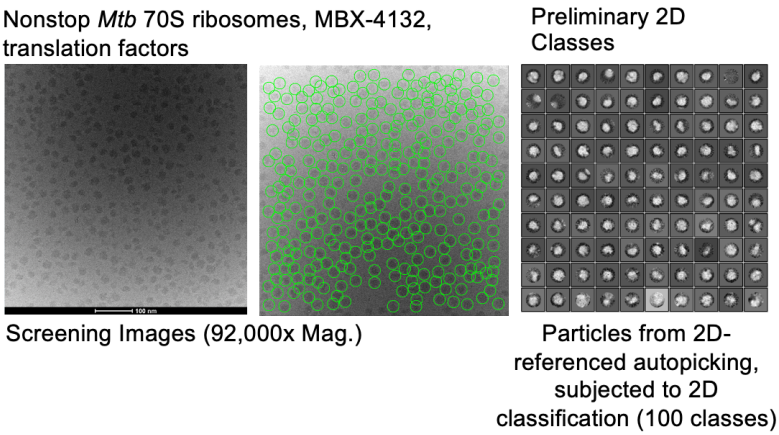

Fig. 1. Preliminary data for Sub-Project 1b.

Sub-Project 2: Regulation of bacterial protein synthesis. For this sub-project, all materials have been cloned, purified, assembled into functional complexes, and biochemically tested. Here, we will investigate two key questions: 2a) how are tRNAs modified by conserved enzymes that recognize both shape and sequence of the tRNA, and 2b) how do mRNA-tRNA mispairings activate the post-peptidyl quality control pathway that result in a loss of ribosome fidelity? Preliminary data for 2a is shown in **Fig. 2**. We have grids made of the complex for 2b (not shown). We solved a version of this complex via X-ray crystallography (without RF2/RF3) that was recently published (PMID:36924943).

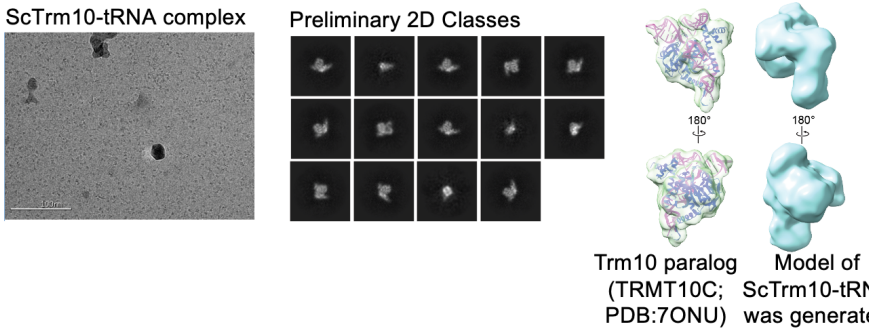

Fig. 2. Preliminary data for Sub-Project 2a.

Sub-Project 3: Ribonucleases activated to inhibit protein synthesis. For this sub-project, all materials have been cloned, purified, assembled into functional complexes, and biochemically tested. Here, we will investigate 3a) RNase R bound to the ribosome and 3b) MazF-mt9 ribonuclease bound to a specific tRNA, with both ribonucleases normally activated during stress. Preliminary data for 3a is shown in **Fig. 3**. We formed this complex using a catalytically inactive RNase R variant that still binds to ribosomes. For sub-project 3b, we have purified a MazF-mt9 and tRNA and have identified a tRNA mutant whereby MazF-mt9 does not cleave but still binds. This complex is ~113 kDa.

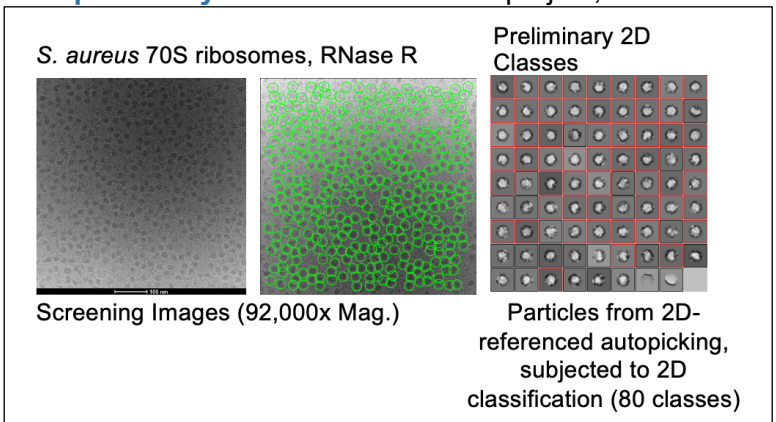

Fig. 3. Preliminary data for Sub-Project 3a.