Supplementary Information - Methylation of conserved 23S rRNA adenosine (A2503), is a low-fitness-cost critical determinant of antibiotic resistance to many classes of antibiotics including oxazolidinones. This nucleotide is located in the peptidyl transferase center and is methylated by the Cfr enzyme. High-resolution structures of Cfr-modified *E.coli* ribosomes, as well as structures of Cfr-modified ribosome with several novel oxazolidinone antibiotics are needed. Because structural difference conferred by methylation is small, the primary goal is the collection of high resolution data sets, acquired on Titan Krios instruments.

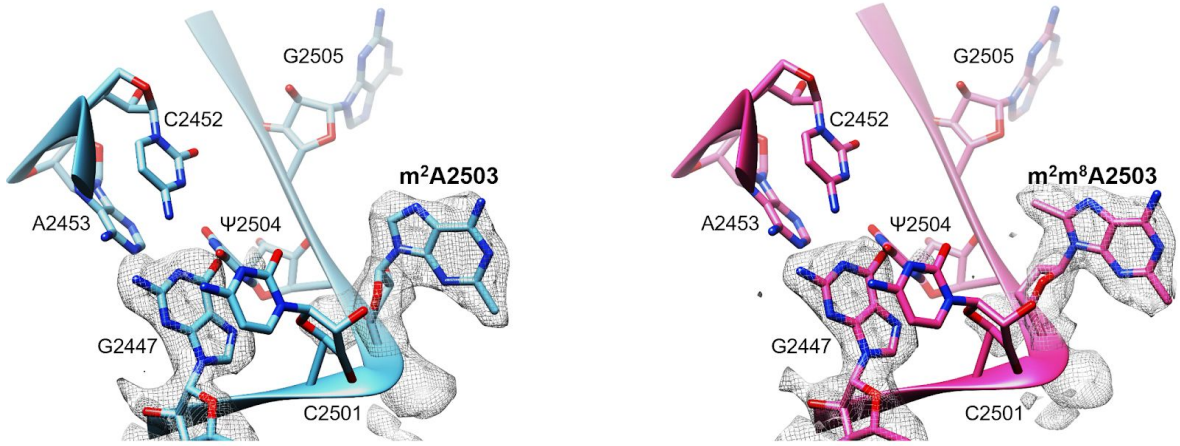

Figure 1 - Detail of the bases (including methylated A2503) in the peptidyl transferase center (PTC). Preliminary structures solved over the course of this project.

Preliminary Data and Sample Verification - Several preliminary oxazolidinone-bound ribosomes have been characterized, including a 50S subunit refined to 2.21 Å resolution.

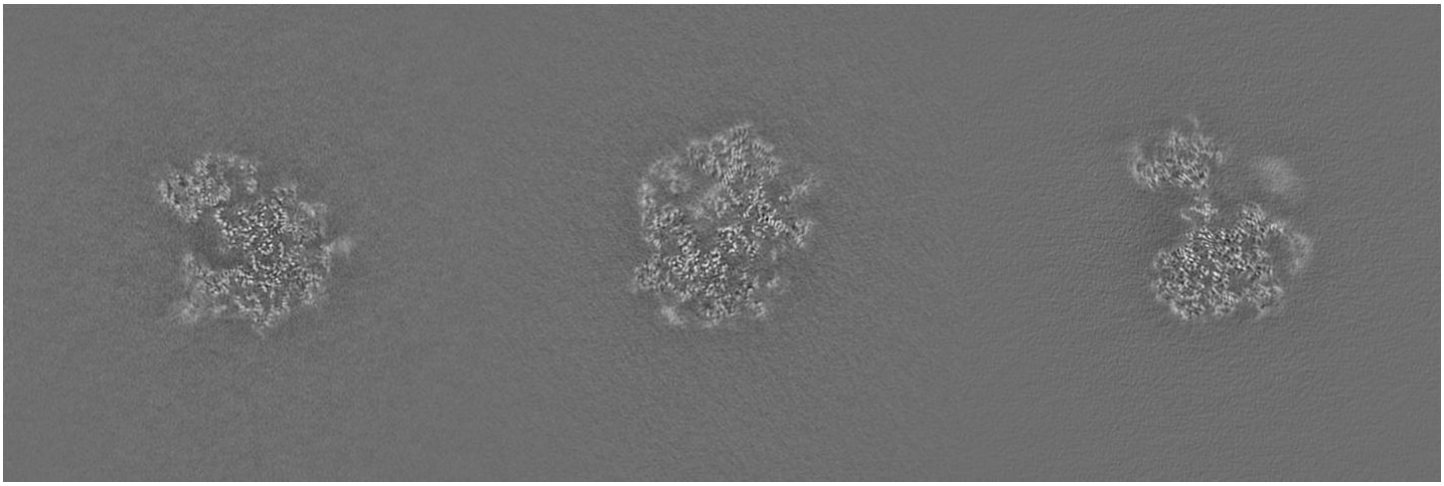

Figure 2 - Preliminary data - Orthogonal bisections of an inhibitor-bound 70S ribosome, with tRNA present, undergoing reconstruction in cisTEM (3D reconstruction currently at 2.55 Å resolution, processing still underway). Density for the oxazolidinone was observed. Two days of collection on a Titan Krios equipped with a GIF and a Gatan K3.

Proposed Experiments - The biggest obstacle in this project is scope access. The sample preparation and biochemistry have been worked out in detail, but insufficient access to Titan Krios instruments that can produce high quality data is the major hindrance. To further the aims of this project, high resolution maps which can resolve the A2503 methylation clearly in the presence of multiple oxazolidinone inhibitors are needed. Preliminary data has well-established the necessity of such resolution. We have several samples of frozen and screened, inhibitor-bound ribosome samples ready for data collection.