Supplementary Information - High-throughput CryoEM methods, as exploited to support this two-pronged approach to studying antibiotic resistance, are ideal as it is possible to achieve sample preparation, data collection, and 3D reconstruction within one week. With sufficient microscope access, iterative rounds of development provide true, rapid structure-based drug design. With sufficient synthetic chemistry support, such an approach could inform and be applied to other antibiotics that currently have limited clinical value.

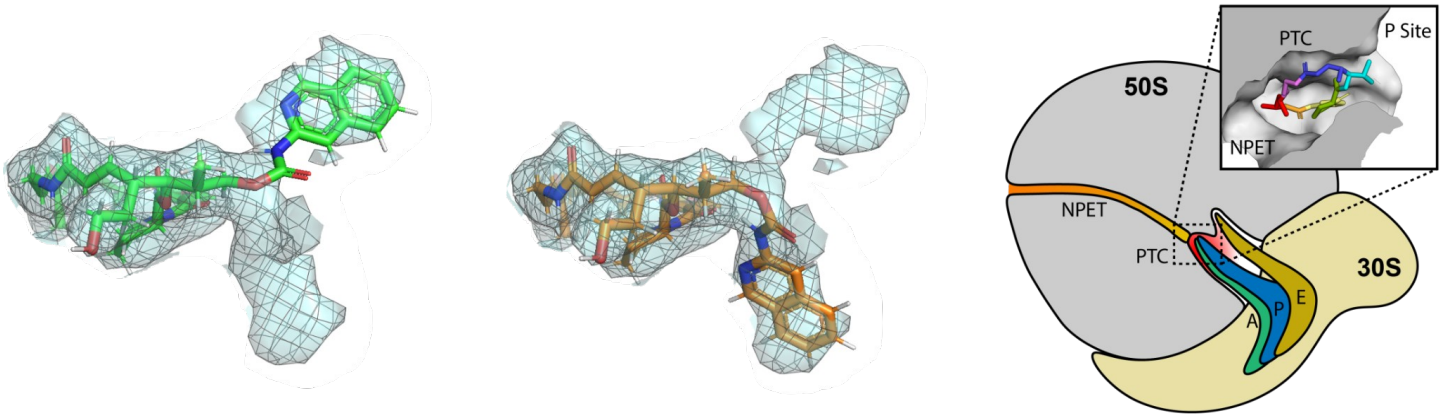

Figure 1: Streptogramin antibiotic that exhibits two distinct poses (green and orange). This is one of several examples of our novel streptogramins that exhibits multiple conformations when ribosome-bound. Improved modeling of multi-conformer antibiotics will bolster our understanding of the significance of these alternative states. We will incorporate this information into future design antibiotic cycles.

Preliminary Data and Sample Verification - Several preliminary oxazolidinone-bound and streptogramin-bound ribosomes have been characterized, including 50S, 70S, methylated 70S, and complexes stalled during translation. In our current studies, we are paying close attention to multiple conformations of bound ligands. We are also attempting to link multiple ligands that bind proximally within the ribosome peptidyl transferase site and nearby tRNA sites.

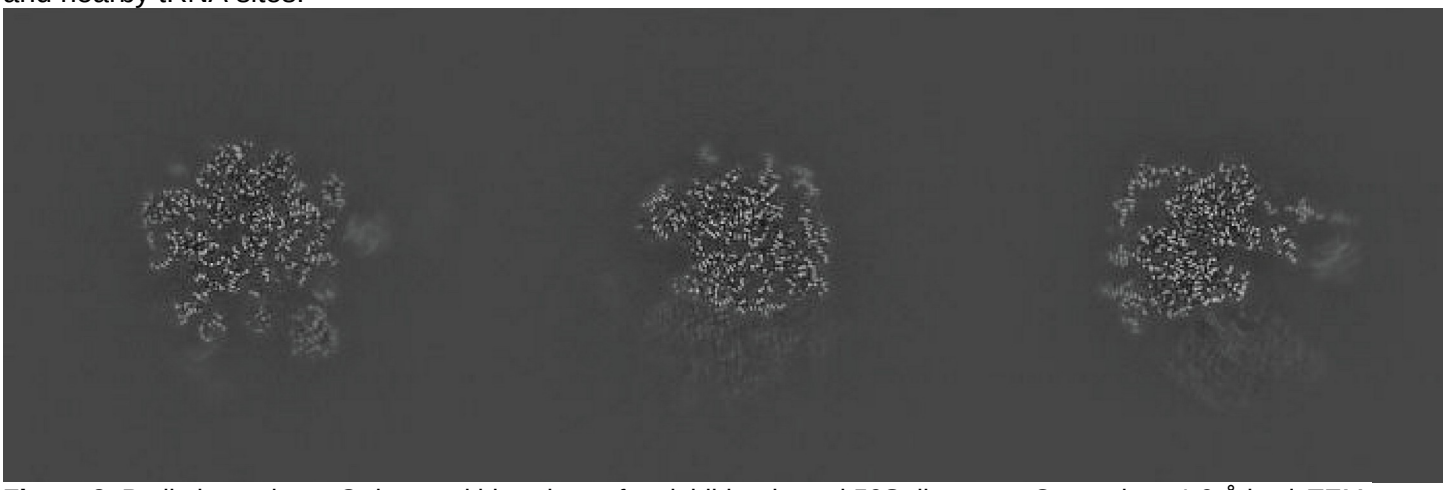

Figure 2: Preliminary data - Orthogonal bisections of an inhibitor-bound 50S ribosome. Currently at 1.9 Å in cisTEM, density for the streptogramin analog was strong. Very slight occupancy of the small subunit observed; will be addressed with masking. Two days of collection on a Titan Krios equipped with a GIF and a Gatan K3.

Proposed Experiments - The biggest obstacle in this project is microscope access. The sample preparation and biochemistry have been worked out in detail, but instrument access remains the major hindrance. To further the aims of this project, long collections are desired to improve particle counts and our ability to subset our data. We have several samples of our next-generation inhibitors bound to ribosomes, frozen, screened, and ready for data collection, as well as previous streptogramins that display multiple conformations in their maps.